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NDB(CF)-55 E 201
Epidemiological Aspects of ALS/PD Complex on Guam

NDB(CF)-59 E 604
Electroencephalographic Studies on ALS-PD Cases on Guam

NDB(CF)-60 E 705
Population Survey and Case-Finding Study of ALS-PD Complex in the Caroline Islands

NDB(CF)-60 E 715
Survey of Diabetes Mellitus and Gout in Guam

NDB(CF)-61 E 832
Studies on the Natural History of Multiple Sclerosis

NDB(CF)-62 E 953
A Serological and Hematological Genetic Survey of Chamorros in Guam

NDB(CF)-62 E 957
Pathological Features of Amyotrophic Lateral Sclerosis and Parkinsonism-Dementia Complex, Both Endemic Diseases on the Island of Guam

NDB(CF)-62 E 959
Further Studies on the CA_1C Variant of Carbonic Anhydrase Enzyme

NDB(CF)-62 E 961
Five Year Registry Study of ALS-PD Cases of Guam

NDB(CF)-62 E 962
Glucose-6-Phosphate Dehydrogenase Deficiency and Other Sex-Linked Traits

NDB(CF)-62 E 964
Electromyographic Studies on ALS and PD

NDB(CF)-63 E 1090
Pedigree Studies in Umatac

NDB(CF)-63 E 1091
Serological and Hematological Investigations among the Trukese in Micronesia

NDB(CF)-63 E 1092
Dermatoglyphic Evaluation of the Chamorros and Carolinians

NDB(CF)-63 E 1093
Blood Group Investigations on the Carolinians and Chamorros of Saipan

NDB(CF)-63 E 1094
Protein Electrophoresis of CSF and Serum of ALS-PD Cases and Controls of Guam

NDB(CF)-63 E 1095
Virus Isolation and Antibody Studies in CSF and Serum of ALS Cases of Guam

NDB(CF)-63 E 1096
Virus Isolation Studies in Brain Tissues of ALS-PD Patients of Guam

NDB(CF)-63 E 1101
Clinical Aspects of Diabetes Mellitus in Guam

NDB(CF)-63 E 1103
Neurological Diseases Other Than ALS-PD on Guam

NDB(CF)-63 E 1104
Measles and Other Virus Antibody in Patients with Multiple Sclerosis

NDB(CF)-63 E 1106
Screening of Edible and Medicinal Legumes for Neurotoxic Constituents

NDB(CF)-63 E 1107
Food, Medicinal, and Toxic Plants of Mitogawa, Japan

NDB(CF)-63 E 1108
Study of Thiaminase and Heat Labile Anti-B_1 Metabolite in "Zenmai" (Osmunda japonica Thunb.)

NDB(CF)-63 E 1109
Study of Toxic Fungi in Cycad and Other Plant Material Used for Human or Animal Food in Various Areas of the World

NDB(CF)-63 E 1110
Preliminary Study of Plants from the Island of Guam: Food, Medicinal, and Toxic

NDB(CF)-63 E 1111
Neurotoxic Effects from Consumption of Cycad Plant Material

NDB(CF)-63 E 1112
Chemistry of the Cycad

NDB(CF)-63 E 1113
Effects on Animals Following Administration of Cycad Plant Material and of Toxic Compounds Isolated from Cycads

NDB(CF)-63 E 1114
Effects of Fresh Cycad Kernel Meal on Open Wounds

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NDB(CF)-63 E 1118
Epidemiological Studies of Multiple Sclerosis in Winnipeg, Manitoba, Canada

NDB(CF)-63 E 1119
Investigation of an Outbreak of Keratoconjunctivitis in La Paz, Bolivia

NDB(CF)-63 E 1123
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NDB(CF)-63 E 1124
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NDB(CF)-64 E 1247
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NDB(CF)-65 E 1248
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NDB(CF)-65 E 1249
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NDB(CF)-64 E 1250
The Reaction of the Nervous System to Cryptococcal Infection. An Experimental Study with Light and Electron Microscopy

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Amyotrophic Lateral Sclerosis, Histopathological Considerations

NDB(CF)-65 E 1252
Reaction of Ependyma to Cryptococcal Polysaccharide Implantation

NDB(CF)-64 E 1253
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NDB(CF)-64 E 1254
Comparison of Glaucoma Response Following Steroid Provocation among Monozygous and Dizygous Twins

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NDB(CF)-64 E 1257
U. S. Mortality Experience with Malignant Neoplasms of the Eye

Special Projects Branch
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Perinatal Research Branch, Summary Report

NDB(CF)-57 PR/ID 402
Sero-Epidemiology of Infectious Diseases in the Collaborative Study on Cerebral Palsy, Mental Retardation, and Other Neurological and Sensory Disorders of Infancy and Childhood

NDB(CF)-61 PR/ID 835
Clinical Investigations in Human Volunteers and Other Populations of Virus Effects and Production of Prototype Human Antisera and Vaccines

NDB(CF)-62 PR/ID 972
Experimental Animal, Tissue Culture, Histopathological and Serological Investigations of the Role of Viruses and Other Micro-Organisms in the Perinatal Period

NDB(CF)-65 PR/ID 1238
Isolation of Infectious Agents from Tissues and Chromosomal Studies

NDB(CF)-63 PR/OC 1144
An Instrument for the Conduct of a Retrospective Study of Seizures, Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood

NDB(CF)-63 PR/OC 1146
A Commentary on the Appropriateness of the Use of Certain Tabular Data, for Formulating Generalizations Concerning Populations in the Same Cities As Those in Which the Collaborative Study on Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood Is Being Conducted

NDB(CF)-65 PR/OB 1268
Methods and Problems in the Epidemiologic Study of Perinatal Data (Yale Report)

NDB(CF)-65 PR/OB 1269
Fifty Years of Research on Mammalian Reproduction

NDB(CF)-63 PR/PN 1153
The Relationship of Birth Order to the Apgar Score of Twins

NDB(CF)-63 PR/PN 1154
Apgar Score - An Index of Infant Mortality

NDB(CF)-63 PR/PN 1155
Apgar Score - An Index of Infant Morbidity

NDB(CF)-63 PR/PN 1156
Neonatal Hyperbilirubinemia and Subsequent Neurological Sequelae

NDB(CF)-63 PR/PN 1159
Aplasia of One Umbilical Artery

NDB(CF)-63 PR/PN 1161
Prematurity

NDB(CF)-63 PR/PN 1162
Apgar Scores

NDB(CF)-63 PR/PN 1163
An Investigation into the Relationship between Congenital Heart and Great Vessel Anomalies and Selected Perinatal Factors as Recorded in the Collaborative Perinatal Research Project

NDB(CF)-63 PR/PN 1164
Early Signs of Neurological Abnormality in Newborn Infants

NDB(CF)-63 PR/PN 1165
Clefts of Lip and Palate as Indicators of Perinatal Morbidity

NDB(CF)-65 PR/PN 1270
Toxoplasmosis: Serological and Clinical Studies

NDB(CF)-65 PR/PN 1271
Maternal Rubella and Pregnancy Outcome

NDB(CF)-65 PR/PN 1272
Hyaline Membrane Disease

NDB(CF)-63 PR/BS 1166
Evaluation of the Gross and Fine Motor Functions of Children Eight-Months of Age in Relation to the Neurological Results of the Examination of the Children at One-Year of Age

NDB(CF)-63 PR/BS 1167
Explorative Study for the Use of a Speech and Language Screening Examination for 3-Year-Old Children in the Home Situation

NDB(CF)-63 PR/BS 1168
Indexes of Socio-Economic Status of Study Mothers in Relation to Outcomes of Pregnancy

NDB(CF)-63 PR/BS 1169
Antecedents and Correlates of Retarded Motor Development

NDB(CF)-63 PR/BS 1170
Socio-Economic Status Groupings and Their Relationship to Illnesses and Disorders as Reported on the OB-3, "History Since Last Menstrual Period," and OB-8, "Repeat Prenatal History"

NDB(CF)-63 PR/BS 1171
An Evaluation of the Offspring of Unwed Mothers in Terms of the Complications of Birth and Delivery, Results on the 4-Month Examination, 8-Month Examination and One-Year Examination, as Compared to a Matched Group of Married Mothers and Their Offspring

NDB(CF)-65 PR/BS 1273
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NDB(CF)-63 PR/EG 1172
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NDB(CF)-63 PR/EG 1180
A Survey of Schizophrenia among the Relatives of Schizophrenic Patients

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NDB(CF)-65 PR/EG 1275
Study of Family Size with Respect to Rh Blood Type and Other Variables

NDB(CF)-65 PR/EG 1276
Sequential Aspects of Occurrence of Spontaneous Abortion in Family Histories

NDB(CF)-65 PR/EG 1277
A Study of the Properties of B. Woolf's Statistic for Combined Analysis of 2 X 2 Tables

NDB(CF)-65 PR/P 1278
Biologic Pattern Data Processing

NDB(CF)-65 PR/P 1279
The Influence of Race and Other Factors on Pulmonary Hyaline Membranes

NDB(CF)-65 PR/P 1280
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ANNUAL REPORT

July 1, 1964 through June 30, 1965

National Institute of Neurological Diseases and Blindness
National Institutes of Health

The Director's Report

During the past year the national research effort in neurological and sensory disorders has continued to show a rapidly accelerating growth. This growth has been evidenced most definitively in the increasing numbers of new applications for research grants, which this fiscal year reached a level of over $25 million. Even more significant, however, is the increased quality and sophistication of the research being proposed, and the evidences of fundamental advance in our knowledge of the structure and function of the complex elements of the nervous system.

Neurological research is increasingly the pursuit of young scientists, the average NINDB grantee being some years younger than his counterpart in other fields. To some extent this reflects the productivity of the Institute's training program, from which capable scientists are now beginning to emerge. Much more, however, it reflects a growing awareness within the scientific community that the instruments and methods are now available with which the complex problems of the nervous system can be productively studied. In addition, the conquest of acute lethal diseases has exposed our failure to deal with the chronic disabling ones. The time has come when the nation can turn its resources toward this neglected area. The report of the President's Commission on Heart Disease, Cancer, and Stroke has amply documented the needs in this area. Its implementation, for which plans have been laid by the Institute over the past several years, will further serve to shift the focus of national concern to the chronically disabled. The Institute is also taking aggressive action to map out special programs for strengthened research efforts for the blind, the deaf, the epileptic, and the brain injured.

The great increase in numbers and quality of new grant applications has provided the opportunity for a high degree of selectivity in the development of the Institute's program.

In working toward its categorical objectives, the Institute must keep in mind both short-term and long-range goals. For the solution of certain disease problems, clearly evident remedies are at hand. In some instances, only the refinement or modification of existing knowledge or methodology is required to meet the specific need. This would appear to be the present situation, for example, in the prevention of blindness, deafness, and brain injury due to rubella. The virus has been isolated. An intensive exploitation of the known methods of vaccine production carries a reasonable expectation of success.

For the solution of many other disease problems, however, such clearly evident approaches are not at hand. More will be necessary than the exploitation of current knowledge. In such areas a further extension of our understanding of the essential nature of the problem will be necessary before an effective approach to its solution can be achieved. This is the case, for example, with multiple sclerosis. Studies over the past few years tend to rule out a predominantly genetic basis for this disorder, and to suggest a role for some autoimmune reaction. However, it is not clear whether there may be a viral agent, some climatic factor, or an unknown toxic or nutritional element playing a part. Wide explorations of these and other presently unsuspected factors in this disorder are necessary before leads on which to undertake the development of specific remedies can be recognized.

It is a responsibility of the Institute to develop and support research having both short-term and long-range potential; to encourage projects of applied research when they carry a reasonable prospect of success; and at the same time, to support a wide range of less specifically relevant research directed toward deepening and broadening our knowledge of the normal and abnormal function of the nervous and sensory systems, and of the processes which damage them.

To maintain an appropriate program balance within these several types of projects, the Institute must maintain a close surveillance of research in its field of responsibility. Such surveillance is of direct value to the Institute staff and its Advisory Council. In addition, the development of an information program, through which such information is made directly available to the scientific community, is one of the most effective means of assuring that program will parallel scientific opportunity.

The increasing strength of the Office of the Director, NINDB, which in the past two years has been augmented by the addition of three senior staff members, has permitted the Institute progressively to strengthen its program analysis and program planning activities. These fall in several categories: the development of a program analysis unit equipped with data processing facilities within the direct operation; the creation of a network of scientific information centers based in university environments of scientific excellence and unusual library capability; and the establishment of special Council subcommittees charged with the responsibility to review in depth a defined area of Institute responsibility. The creation this year of two Subcommittees, one on Vision, and the other on Speech, Hearing and Human Communication, reflect the Institute's concern for these important areas.

Faced with limitations of research and of training funds, the Council has considered methods for establishing priorities among competing programs and projects. There has been great reluctance to modify the priorities established by Study Sections, since these priorities provide the best available index of the scientific worth and potential contribution of the applications received. The Institute's concern with categorical objectives expresses itself instead in efforts to encourage the development of high quality projects in selected areas of program need.

In addition to direct staff activities, the Institute this year has supported the following conferences and workshops, designed to highlight research developments

and opportunities. Through direct operations, the Institute sponsored scientific meetings on headache; rubella virus; multiple sclerosis; epilepsy; medical information centers; head injury; brain tumor; television ophthalmoscopy; radiation diagnostic aids; metabolic disorders in the newborn; and latent, slow and temperate virus.

The Institute also awarded extramural grants for conferences on glaucoma; vascular disorders of the eye; biophysics of axons; the definition of blindness; mutant mice with neurological diseases; information processing in sight-sensory systems; brain mechanisms underlying speech and language; nomenclature of head trauma; and a planning conference for the Second Pan-American Congress of Neurology.

In certain areas of special need, specific programs have been established as part of the direct operation. Thus, for example, a recognition of a need for greater concern for a possible role of "slow" and "latent" viruses in central nervous system disease led to the development five years ago of a virus laboratory at Patuxent, the organization of a cultural exchange mission to the Soviet Union, and this year an important conference at Bethesda on this topic, which is now exciting widespread interest. Within the collaborative and field area, similar efforts are in progress to provide increased emphasis in two other important areas of concern, namely, epilepsy and head injury.

The increased strength of the Institute's senior staff has also made possible an important sharing of responsibility in the decision making process of the direct operation. The organization of a "Scientific Directorate" has formalized this step. Increasingly, activities within the direct operation, especially those involving contract mechanisms, are being subjected to a scientific and policy review closely paralleling that of the extramural program. The Institute is receiving increasing numbers of requests from industry for the support of contracts. Many of these requests relate to the development of new diagnostic, therapeutic, or research instrumentation. There exists within industry a tremendous potential contribution to medical research, yet the Institute has neither the funds nor the staff to take advantage of this opportunity.

As indicated above, the Institute's staff has been augmented during the period of this report by the appointment of Dr. Eldon L. Eagles as Assistant Director, Dr. William F. Caveness, Associate Director for Collaborative and Field Research, and Dr. Edgar A. Bering, Jr., Special Assistant for Program Analysis. Dr. Ronald Myers has been appointed Chief of the Laboratory of Perinatal Physiology in Puerto Rico.

The growth of the Institute's program is depicted on the attached charts. Its present magnitude may be appreciated when one considers the following summary of its activities.

At the NIH Clinical Center, NINDB's programs of Neurosurgery, Medical Neurology, and Ophthalmology had 631 inpatients during the year and 1721 outpatients. There were 136 major surgical procedures and 47 minor; 296 muscle biopsies; 1749 EEG's plus myelograms, pneumo-encephalograms, and cerebral angiograms. Many of these procedures were performed in collaboration with the other Institutes at NIH and other Government agencies.

Clinical Center neurosurgical programs related particularly to cerebral trauma, epilepsy, and developmental defects. Medical neurology's program continued the classification of the myopathies; developed a classification for the periodic paralyses and non-dystrophic myotonias; studied the immunologic aspects of myasthenia gravis; and conducted studies relating to ALS, brain tumors, and cerebrovascular disease. Ophthalmology programs related to retinal disorders, uveitis, intraocular pressure, corneal infections, and degenerative disorders, lens changes, and ocular changes in systemic disorders.

In the intramural's 10 labs and branches, there were 192 research projects this past year compared with 170 during the previous year. One hundred and one of the 192 projects this year involve collaboration with other labs and branches within NINDB, NIH, and outside organizations.

The Institute's Extramural Program included 1615 research grants funded with $50.9 million. Of these, 260 were new grants and 1355 continuing programs. Twenty-four clinical centers (16 neurological and 8 sensory) are being supported. There are now 248 graduate training programs supported with $12.4 million. Additionally, 200 special fellowships, 63 postdoctoral fellows, and 75 research career development awards were supported by the Institute.

The following statements briefly review the Institute's approaches to each of its major areas of defined responsibility.

NEUROLOGICAL DISORDERS OF INFANCY AND CHILDHOOD

A large proportion of these disorders, which often manifest themselves by mental retardation, cerebral palsy, deafness, or blindness, have their origin prior to birth or immediately surrounding it--the so-called perinatal period. Inheritance, prematurity, metabolic disorders, infections, or physical injury, at or after birth all are known to contribute to these neurological disorders, but in a large majority of cases, medical science cannot yet with certainty define the specific causes. To meet this challenge, the Institute has developed a broad program of clinical investigation, "The Collaborative Perinatal Project," in which the circumstances and events of the perinatal period are being observed and recorded in 50,000 women and these data are being correlated with the development of their children as determined by a series of followup examinations. This year it has been decided that this followup is to continue through the twelfth year of life--long enough to evaluate the school performance of the offspring.

Data from this project are now being analyzed at an increasing rate. For example: General anesthesia of the mother during Cesarean delivery is related to poorer responses at birth of some Cesarean newborn.

More than 17 percent of pregnant women have no blood-borne protection against German measles (rubella), an important cause of birth defects.

A more than twofold increase not only in perinatal mortality but also in neurological abnormalities is observed among children born to older-age mothers--those 40 years of age or older.

Toxoplasmosis, a protozoan infection, may be more important as a factor in perinatal damage than had been thought. Preliminary data from the Project suggests an incidence of 1 affected child per 1000 to 2000 born.

The Study is defining more clearly the pregnancy complications which raise the chances that a child eventually will show signs of mental deficiency.

All children in the Collaborative Project are still under observation and the majority of them are only 2 years of age. About 2 percent of the children examined at 1 year of age showed neurological or mental abnormalities.

The clinical investigations within the collaborative project do not permit experimentation, yet many of the hypotheses developed in this study require experimentation for their definitive solution. The Laboratory of Perinatal Physiology, in Puerto Rico, has been established to provide this opportunity. More than 1000 monkeys are now available in that facility for the study of the impact of perinatal factors in the developing nervous system. Under the new chief of the laboratory, Dr. Ronald Myers, investigations of the impact of compression of the umbilical cord, asphyxia, intoxications, and modifications of neural behavior occasioned by specific brain damage are being investigated.

Another major focus in the Institute's study of neurological and sensory disorders of infancy is the study of specific biochemical disorders of the brain. For example, the recent finding by Institute scientists that a specific enzyme deficiency may cause Gaucher's disease is noteworthy. Babies with this disease often become mentally retarded if they survive. Most die before the age of two. It may now be possible to devise a means to correct the biochemical defect--a lack of effectiveness of an enzyme which normally processes a sugar and fat molecular complex called "glucocerebroside."

A comprehensive clinical, genetic, and laboratory study of children suffering from organic brain syndromes is being conducted by the Institute's Section of Child Neurology. It has been observed here that, while some patients with cerebral palsy are also mentally retarded, others only appear to be mentally subnormal because they are unable to communicate adequately.

Essential to all these investigations is a deepened understanding of the fundamental structure and function of the developing nervous system. Progress continues in quantitative and qualitative analyses of the outer layers of the brain, including new knowledge of the density and patterns of brain nerve cells and their connection. At present, brain damage is irreversible. Until fundamental knowledge of the brain is acquired, there is little prospect of repair of destroyed tissues of the brain. Investigations within the Institute's Laboratory of Neuroanatomical Sciences are directed toward increasing our knowledge of the factors influencing growth patterns in the brain.

In other investigations pathological evaluation of the brain of carefully studied children is attempting to link pathological patterns of brain damage to previously observed symptom complexes of cerebral palsy or mental retardation.

The ultimate aim of the Institute's program is the prevention or treatment of the many disorders of the nervous system that may lead to mental retardation

and cerebral palsy. In addition, through the study of neural functional patterns, efforts are being made to ameliorate the devastating results of such injuries when they occur.

INFECTIOUS DISEASES OF THE NERVOUS SYSTEM

Extensive research is conducted or supported by the National Institute of Neurological Diseases and Blindness to identify and control infectious agents causing neurosensory disease.

With the demonstration in animals of slow-developing, chronic, degenerative viral diseases, such as scrapie in sheep, the Institute seeks to find parallels in human disorders such as kuru, amyotrophic lateral sclerosis, and subacute sclerosing encephalitis. Animal studies of the "slow" viruses are being conducted at Institute laboratories at Patuxent, Maryland, and related studies are under way in New Guinea and Guam.

The Institute sponsored a delegation to the Soviet Union to study infections of the nervous system and Russian hypotheses about them. A main interest was a Soviet report that a virus causes amyotrophic lateral sclerosis. However, the delegates were not convinced of the presence of a transmissible agent in material submitted to them for examination.

An Institute-sponsored Workshop Symposium on Slow, Latent, and Temperate Virus Infections was held in December 1964 to further explore the possibility of a viral etiology for chronic neurological diseases.

Another Institute area studying infections is the Collaborative Perinatal Project. Particular emphasis has been given research on German measles (rubella). When acquired by a woman during the first 3 months of pregnancy, this infection may cause defects in her infant. The Project is evaluating the efficacy of rubella gamma globulin, and is attempting to develop a safe and effective vaccine against rubella. Other Project research on perinatal infections has provided evidence, for example, that salivary gland virus and toxoplasmosis infections are more widespread in mothers and newborn than had been realized. Both of these agents can damage or destroy the fetus or newborn.

Institute grants, amounting this year to $378,000, support research on infectious diseases of the nervous system. Some examples are work on the measles effect on the myelin sheath protecting nerves; epidemiological studies of an epidemic of Eastern equine encephalitis, to determine the extent of neurosensory sequelae; research on viral keratoconjunctivitis and on uveitis of infectious nature; and studies of experimental demyelination with diphtherial toxin in cultures.

MULTIPLE SCLEROSIS

Multiple Sclerosis (MS), a disorder involving the disintegration of the myelin sheath around nerve fiber tracts in the white matter of the brain and spinal

cord, remains a baffling disorder for which no cure nor effective therapy has been devised.

Suspected but still unproved are various possible causes or triggering mechanisms. Therefore, research in MS is wide-ranging, including studies of the chemical and physical processes related to myelin formation and its destruction. Other major research pathways are related to the epidemiology of the disease, and its possible viral etiology and autoimmune implications.

Intensive investigations of the possible environmental factors responsible for the disorder continued. A followup study of a previous epidemiological survey of the disease in New Orleans and Winnipeg, Canada, added to comparative data on prevalence and incidence trends. Other geographic or climatic aspects of the disease are being investigated in cooperation with the U. S. Weather Bureau, the U. S. Department of Commerce, the National Academy of Sciences, and the National Research Council.

The previous finding in multiple sclerosis patients of two types of serum antibodies to measles was thoroughly investigated in pursuit of factors which may explain a virus etiology.

Because loss of patches of myelin is a key problem in MS, intensive research effort was directed toward gaining a clearer understanding of its anatomy and chemistry, with the hope of finding new clues to the fundamental biochemical processes involved in the formation and destruction of myelin.

Tissue culture technique now permits direct observation of the formation and destruction of myelin. This year Institute investigators examined the effects of various enzymes and biochemicals on the processes of myelination and demyelination.

The well-known experimental model of MS--allergic encephalomyelitis (EAE)--created by injecting emulsions of brain and spinal cord tissue from other animals in a suitable media--was subjected to various research techniques, to ascertain whether immunologic abnormalities exist in EAE and MS. The theory that in MS, some of the patient's own nervous tissue proteins are altered to act as antigens and that the body may create antibodies to these proteins which, as in the EAE animals, demyelinate human nerve tissue, remained under active investigation.

The immunofluorescent antibody technique was used to localize the sites of antibody activity in the central nervous system. Because the gamma globulin fraction of proteins is known to be partially converted to form antibodies, careful studies are under way of this fraction in the blood fluid and cerebrospinal fluid in MS patients and in normal controls. The gamma globulin patients with MS can be divided into two distinctive immunologic patterns, while, in normal controls, only one pattern exists. The nature of the abnormal protein fraction in the MS patients is now being elucidated.

Among significant program developments in the year was Institute joint sponsorship with the National Multiple Sclerosis Society of the first in a series of scientific workshops.

Efforts to evaluate therapy moved ahead with the initiation of a cooperative double-blind study to investigate the effects of ACTH on a short-term basis.

AMYOTROPHIC LATERAL SCLEROSIS (ALS)

Amyotrophic lateral sclerosis (ALS) is a progressive disease characterized by muscle weakness due to degeneration of motor cells in the spinal cord and brain. Its cause is completely unknown. Therefore, when this disorder was found to be 100 times more prevalent in Guam than in the United States, a small research laboratory was established in that environment. Within this facility, much additional information has been collected from patients and autopsy studies, also, it has been established that there is on Guam another closely related neurological disease--a form of Parkinsonism. The relationship between these two neurological syndromes is still under investigation. A long-term epidemiological study of the Guam population seeking to distinguish a genetic from an environmental factor is in progress.

Because of the suspicion that an environmental factor was operative in this population, thorough investigation for toxic agents or nutritional deficiencies was instituted. Analysis of brain specimens of patients dying with ALS are being carried out for essential trace metals including manganese, copper, tin, aluminum, titanium, lead, and silver. It now seems unlikely that the cycad nut, an exotic source of food in this population, is responsible, although it has been demonstrated to contain a potent carcinogenic agent.

A new study is focusing attention on the analysis of urine specimens for metabolic products in ALS patients and controls. This approach, which has received little attention in the earlier studies of motor neuron disease, may provide further insight into a possible metabolic defect in ALS. It now appears to hold special interest since preliminary studies reveal that the Marianas population has an unusual prevalence of abnormally high uric acid levels.

A second possibility is that ALS is a virus disease. Current investigations at the Bethesda laboratories, and at the special laboratory for the study of chronic viruses established in collaboration with the Fish and Wild Life Service at Patuxent, Maryland, are directed toward a search for viruses in this and other diseases. Electron microscopy is also being used in an attempt to isolate virus particles in the unique granular areas of the nerve cell (granulovascular bodies) found in ALS patients at autopsy. Soviet reports claiming the transmission of ALS from patients to monkeys were investigated by the cultural exchange mission sent to the Soviet Union this year and material was brought back from Russia for investigations. Animal inoculation studies using this material are being carried forward to investigate the characteristics of these viral agents.

Within the intramural program, an intensive review of the literature and a critical analysis of the problem of ALS has been completed. Fundamental investigations of the chemistry and fine structure of the anterior horn cell are being developed.

PARKINSON'S DISEASE

The focus of the Institute's research effort the past year has been centered on several crucial aspects of the problem: the development of new drugs for the relief of symptoms; the improvement of surgical techniques to control tremor; the search for abnormalities of brain chemistry (metabolic defects); an evaluation of hereditary factors; an investigation into the role of viruses as a cause of chronic epidemic encephalitides; and the study of the anatomical, physiological, and pathological basis of the disease.

Intensified basic research on the mechanisms and sites of action of drugs indicated a higher than normal concentration of certain enzymes in the nerve cells of the thalamus. Now additional plans are underway for laboratory and clinical investigation into the electrophysiological and behavioral effects of a variety of compounds, including many of the drugs currently employed in the treatment of disorders of involuntary movements. Through use of multiple electrodes introduced into certain areas of the brain, it is anticipated that localization of major sites of actions of drugs of proven value will be possible, and will provide a pattern for development and evaluation of experimental drugs.

Reports of several grantees have suggested that a disturbance in catecholamine metabolism may play an important role in the development of Parkinsonism, and special attention is being turned to the content of the substantia nigra, a plate of pigmented cells at the base of the brain. Neuroanatomical studies of efferent fibers carrying impulses from the substantia nigra indicate that degeneration here interrupts transmission of signals to the outer layer of the brain. And new findings concerning inheritance of metabolic defects, gave impetus to investigation into a possible inheritance of susceptibility to Parkinson's disease.

Another 1965 approach is opening from data presented at an Institute-supported International Workshop on slow-acting types of viruses which attack the central nervous system of animal and man and may be responsible for various chronic neurological disorders. As part of the study of viral transmission, the Institute's facility at Patuxent is using material from patients with a variety of neurological disorders to inoculate animals for prolonged observation.

Highly encouraging findings have been made this year in clarifying the manner in which the disease acts on the individual and in better controlling its symptoms.

One of the major accomplishments for the year has been the opening of the Institute-supported Parkinson's Disease Information and Research Center in New York. This Center is collecting current and past research findings, both American and international. It provides library service, publishes periodic reviews, and conducts symposia and meetings. Here, professional members of the staff evaluate all available reports and serve as a principal source of information on current research. It also conducts a closely integrated program of basic and clinical research and serves as a training center for research personnel.

As part of the Center's program, a new workable diagnostic classification has been developed to obtain statistical models of Parkinsonism. The categories are: Parkinson's Disease, Post-encephalitic Parkinsonism, Other Central Nervous System Disease with some Parkinsonian features, Symptomatic Pseudo-Parkinsonism, Essential Tremor, and other Dyskinesias.

This year at a Center-sponsored international conference devoted to study of the thalamus, highly promising findings were reported by an NINDB grant-supported team. These investigators converted electrical signals from the brain into sound pulses to guide the surgeon through the previously uncharted areas of the brain. The tiny electrode probes are moved around in the thalamus until the signal sounds have the same frequency as the tremors, identifying the exact area where destroying a minute amount of the tissue can control the tremor.

DRUG EVALUATION

The development and evaluation of new therapeutic agents is of major interest to the Institute. Drugs under study for the treatment of several types of strokes include anticoagulants, anticholesteremic compounds, antihypertensive agents, fibrinolytic substances, and vasodilators. Over 450 Institute grants include support for the development or evaluation of therapy of neurological disorders.

Adrenocorticotropic hormone (ACTH), because it modified the immune reactions of the body, has often been used in treating myasthenia gravis. An Institute-supported collaborative study evaluated the effectiveness of ACTH for ocular myasthenia earlier this year. This controlled study showed no evidence that ACTH is effective in producing improvement.

A series of Institute-sponsored workshops have developed methods for clinical evaluation of drug treatment of multiple sclerosis and a pilot study of ACTH has been initiated.

A newly-initiated study of drug-muscle receptor interactions has been undertaken to discover how the neurohormones acetylcholine and histamine produce their effects and what the relationship is between the structure and their stimulant or blocking activity at the nerve-muscle junction. From a similar long-term study, an Institute investigator demonstrated that certain types of drugs enter the muscle fibers and produce an excitation block which is irreversible with available antagonists.

To provide increased emphasis on the study of basic mechanisms of drug action the Institute supports an important center on neuropharmacology to serve as a focal point for comparative evaluation of both old and new drug therapies.

In addition to its search for new drug therapy in treating neurological disorders, the Institute recognizes the need to be constantly alert to possible neurological side effects of drugs used to treat other diseases. NINDB grantees, for example, pinpointed the pathologic changes in the human ear

caused by such antibiotics as streptomycin, which often led to permanent deafness. Institute grantees reported hearing defects in offspring attributable to chloroquine taken by the mother during pregnancy.

Conjunctival and corneal changes are an occasional occurrence following topical therapy with epinephrine preparations for glaucoma. Institute investigators have reported these to be fairly common side effects of chloroquine therapy for chronic diseases such as rheumatoid arthritis.

Toxic retinal degeneration has been associated with phenothiazine tranquilizers used in the long-term treatment of psychotic patients. This year, an Institute grantee disclosed that the localization of chloroquine and phenothiazine compounds in the uveal tract is a property shared by a number of polycyclic aromatic compounds. According to his studies, compounds such as chlorpromazine and chloroquine have an affinity for retinal pigment due to a reaction which binds the drug to pigment.

A workshop is planned in 1965 to consider the risks of side effects on the eye of systemic medication, together with the means of detecting such changes during pre-marketing evaluation of new drugs.

The Institute's training program provided approximately $500,000 for the training of specialists in neuropharmacology.

VISION

During the past year, the Institute supported almost 400 research projects relating to the mechanisms of vision and all the blinding disorders--cataract, glaucoma, amblyopia, infections, accidents, vascular disorders, and the many disorders of the retina and of the cornea.

From these Institute-supported projects last year have come a number of significant findings. In relation to cataract, the use of extreme cold was found to make cataract surgery safer and easier. Removal of the opaque lens can be accomplished more quickly and safely by placing the frozen tip of a probe against the lens until it freezes fast to the probe and can be removed in its capsule.

There have been continued improvements in the treatment of eye infections. This year Institute-supported scientists conducted trials on a more effective drug for herpes simplex keratitis, an infection of the cornea. Successful treatment of one type of uveitis with an antitumor drug called methotrexate was encouraging to Institute investigators.

Experiments with a new method of freezing corneas for corneal transplants may improve chances of restoring vision to victims of corneal scarring. Investigators have also conducted further studies on plastic implants to replace the natural scarred cornea and have introduced a silicone implant to help prevent failure of corneal transplants.

Glaucoma advances from Institute-supported research this past year include strong evidence that all primary glaucoma is inherited as a recessive trait.

Institute intramural and grantee studies relating to the retina contributed to an explanation of the way the eye sees color, helped perfect several methods of treating retinal detachments, and proved that vitamin A in soybean oil improves retinal function in a tapeto retinal degeneration called acanthrocytosis.

Early detection of retinal damage such as that produced by the drug chloroquine was made possible by improvements in the clinical technique of static perimetry.

Progress was made this past year for those suffering from amblyopia. The new technique of "pleoptics" is restoring vision to children and young adults who previously were blind in one eye because of improper utilization of the eye in early childhood.

Within the Institute's Collaborative Perinatal Project, toxoplasmosis was found to occur more frequently than expected. Methods have been developed for the early diagnosis in pregnancy of this condition, which leads to blindness and mental retardation.

Recent statistics demonstrate the increasing importance of vascular lesions of the eye, especially those associated with diabetes. Clinical and pathological investigations are attempting to determine the mechanism through which the abnormal vascularity develops.

The Institute is increasingly concerned with the frequency with which vision is lost through unanticipated side effects of new and old remedies. A variety of such harmful effects have been demonstrated. These include cataract from MER-29, and damage from certain tranquilizing and antimalarial drugs. The Institute is attempting to determine the mode of action of these agents as well as techniques for early recognition of ocular damage.

There has been a serious lack of reliable data regarding the frequency of blindness, and the geographical distribution and etiology of the various blinding diseases. The Institute has developed two programs to overcome this deficiency. The "Model Reporting Area for Blindness Statistics" capitalizes on the already existing state-supported data collection systems. Through the achievement of uniform data collection, reporting and coding systems, the data from the 12 member States may now be collated into a single report. Four more States will be added this year and it is hoped that membership can be extended to cover a major segment of the U. S. population.

More precise epidemiology data are being obtained within the Epidemiology Branch. Field studies are being developed to conduct examinations of selected diverse populations to observe the prevalence of increased intra-ocular tension and other deviations related to blindness.

In order to encourage the application of varied laboratory techniques to the clinical problems presented by the blinding diseases, the Institute has established three clinical research centers specifically for eye research, and another as a segment of a larger center on sensory and perceptual disorders.

Plans have been developed for one of these to serve as the focal point for review and dissemination of scientific information in this field.

To increase the supply of laboratory and clinical scientists to staff these centers, and to man the total program, this Institute this year provided $2,285,000 for training grants in clinical and laboratory vision research and supported 27 special trainees in advanced training.

To provide a focus for review and program planning in blindness research the Council has established a special Vision Research Subcommittee to conduct an intensive survey of this field.

HUMAN COMMUNICATION

Development of normal communication--hearing, language, and speech--has received more concentrated and multidisciplinary attention this past year than at any previous time. During the past year, the Institute made more than 170 grants for some $5 million for individual research projects in disorders of hearing, equilibrium, speech, and other associated functions.

One grant-supported research team developed a new method for using ultrasonic energy for effectively treating Meniere's disease without severely damaging the facial nerve. A new technique for quantitative measurement of airflow during speech has aided physicians in the diagnosis of disorders of the larynx. Current findings resulting from a study of the mechanism within the neuron indicate that the interplay of excitatory and inhibitory post-synaptic potentials is important in localizing the directional origin of sound. Studies comparing brain-injured preschool children with normals have shown that language, conceptual, and perceptual motor functions are less affected than personality traits. Also, Institute grantees have contributed significantly to the development of the new International Standard for audiometers which promises to resolve the confusion resulting from interpretation of audiometric tests.

The research program conducted at the Institute's Bethesda laboratories has resulted in further clarification of the details of structure of the inner ear and has specified more precisely the pathways and terminations of afferent and efferent nerve fibers between the brain and ear. Auditory perception and linguistic function, along with other psychological features, are being studied in clinical patients undergoing temporal lobe surgery.

Three clinical center programs focused on auditory functions are supported by Institute funds. One of these, the University of Chicago Center, concentrates on the clinical investigation of disorders of hearing and equilibrium with special attention to studies of temporal bones of known cases of deafness. The Center at the Central Institute for the Deaf in St. Louis, studies normal auditory functions including parallel studies of communication by vision, touch, and vibration. And the Princeton University Center's program encompasses the investigation of hearing in all its fundamental aspects, not only in humans, but in other mammals and reptiles.

Progress is being made also through the Collaborative Project and its perinatal research laboratories. Factors being investigated include maternal infection with a variety of virus diseases (particularly rubella), drug ingestion, asphyxia at birth, and metabolic disorders.

The Institute's training grants, support of special fellows, and career development awards form an important part of its program to combat communicative disorders. Support also is provided to over 50 training programs at academic institutions. These include otolaryngology, audiology, speech pathology, and sensory physiology in which over 300 postdoctoral students are receiving training.

STROKE

The Institute is supporting basic as well as clinical studies designed to improve the diagnostic accuracy and appropriate choice of treatment for cerebrovascular problems.

In addition, because of the pressing practical clinical problems of diagnosis and patient care, four clinical research centers have been established for stroke: one concentrating on epidemiology and pathology, one on diagnosis and therapy, one on clotting mechanisms, and one on blood flow.

A major obstacle in cerebrovascular diagnosis has been the inability to view directly the total circulation of the brain. Angiography is one of the best diagnostic aids available to "see" through the skull. A new procedure, called the subtraction technique, improves the effectiveness of angiography. It consists of a photographic process utilizing electronic (television) recording devices which can cancel out all X-ray images (including those of the skull) except the opaque material outlining the vascular system of the brain.

A significant number of patients with brain hemorrhage due to the rupture of a cerebral arterial aneurysm (a sac-like swelling of the blood vessel) are found on angiographic study to have not one, but several aneurysms. An Institute-supported investigator has devised highly effective criteria for locating the rupture quickly when multiple cerebral aneurysms are present. By using two techniques, angiography and classical X-ray findings, this investigator found that the diagnostic accuracy was 96 percent.

Radioactive brain-scanning devices now give promise for identifying patients with cerebrovascular disease. One grantee reported on a brain-scan study of nearly 200 patients with cerebrovascular disease. Results indicate that when a scan is abnormal, the neurological deficit is more likely to be permanent. A normal scan, on the other hand, suggests that the patient has a good chance for full recovery.

In order to expedite the necessary development of new diagnostic equipment, the Institute this year has sponsored two workshops designed to bring together the industrial experts and the laboratory and clinical scientists concerned with the use of these new techniques. It also supported an important

international conference on neuroradiology attended by more than 1200 scientists at which these newly developed methods were presented and reviewed by approximately 75 internationally known experts.

The evaluation of preventive and therapeutic measures in stroke is a second important element of the program. One of the Institute's four cerebrovascular research centers has initiated a study to evaluate the use of equine estrogenic hormone (premarin) for patients who have had a single stroke. Another investigative team, using anticoagulants plus a plasminogen activator (streptokinase) in treating progressive stroke, has concluded that streptokinase therapy is contraindicated in the treatment of progressive stroke.

Data from a cooperative study on anticoagulant therapy suggests that anticoagulants are useful on a short-term basis for patients with transient ischemic attacks (incipient "strokes") and progressive strokes. However, it concluded that long-term anticoagulant therapy did not lower the mortality rate once an actual blood vessel block had occurred. On the other hand, one investigator has noted that, under ideal conditions, there is evidence that some patients with completed strokes may be benefited through the use of anticoagulants. This point needs more complete validation.

Twenty-four laboratories are participating in a cooperative study of therapy in intracranial aneurysms and acute subarachnoid hemorrhage. The study is attempting to determine when each of the four most promising treatments for cerebrovascular aneurysms is most applicable. The first major report of this study is being prepared for publication in 1965.

A group of neurosurgeons has developed a method of electrically inducing clots in cerebral aneurysms. This method calls for cutting a burr hole in the skull rather than "turning a bone flap." Guided by copper grids and angiography, a copper needle containing a sharp-pointed positive electrode is thrust into the aneurysm. Then, with the negative electrode located on the skull between the skin and bone, electric current is turned on and the clot-forming process begins. Several angiographs are taken to measure the progress of the clot. When the clot is complete, the darkened area representing the aneurysm disappears from the angiogram.

These special studies are being undergirded by a broad range of grant-supported projects concerned with the epidemicity of cerebrovascular disease; factors influencing cerebral blood flow; the anatomy, pathology, and physiology of the cerebral blood vessels; and factors influencing tissue reaction and the blood-brain barrier.

NEUROMUSCULAR DISORDERS

This general category comprises a number of diseases having as their common characteristic progressive wasting and weakness of muscle, but differing widely in their specific manifestations and essential nature. Since the clinical symptomatology of these various diseases is confusing, the first requirement toward an understanding of these conditions has been the development of reliable means for their differentiation.

This task has been a major activity of the Institute's Medical Neurology Branch for almost 10 years. The complexity of the problem, and the tremendous strides which have been made are depicted by the attached table which summarizes a classification presented this year by the Branch.

It is clear that there are now two closely related approaches to the problem: the pathological and histochemical study of normal and diseased muscle, and the search for systemic deviations of physiology or chemistry which may lead to muscle dysfunction.

A number of specific advances can be reported. Institute scientists have identified four subgroups of muscular dystrophy with structural and biochemical abnormalities at the nerve-muscle junction found in every type. Two of these varieties are now known to derive from specific enzyme defects. A third involves a recognizable muscle abnormality associated with atypical structure in the core of the muscle fiber, leading to the name "central core disease."

A fourth variation, described by Institute scientists during the past year, is a new disease called "nemaline myopathy." This unique muscle-cell structural abnormality is associated with non-progressive weakness and mental retardation. A child with nemaline myopathy symptoms has the clinical picture of a "floppy infant" at the period just after birth and as late as 4 years of age. Muscle weakness and mental retardation persist throughout life, but the disorder is not progressive. Present studies are designed to compare the small, rod-like particles found in the muscle cells of nemaline myopathy patients with samples of pure fetal myosin to help identify the particles and better understand the disease.

Interest in specific biochemical defects in neuromuscular diseases is typified by Institute studies of enzymes normally present in muscle tissue. In muscular dystrophy, the enzyme lactate dehydrogenase-5 is absent or severely diminished. Institute studies were the first to show that a similar deficiency of this enzyme exists in every kind of disease affecting muscle.

An example of successful biochemical studies is the creatine phosphokinase test of blood serum, now being improved for mass testing in patients. This method of identifying carriers of muscular dystrophy has proven 70 to 75 percent effective, according to evidence accumulated from many medical centers both in this country and abroad.

Following reports of successful growth of muscle tissue from dystrophy patients, Institute investigators initiated tissue culture studies. Samples of muscle tissue obtained from muscular dystrophy patients are grown at body temperature in a balanced medium simulating the acid-base components of human fluids and the nutrients available to the muscles. It became apparent that this muscle tissue might grow differently than normal muscle, and a new method of observing the growth characteristics of dystrophic muscle is now available.

Recently, investigators employing similar techniques called "diffusion chambers" to study muscle growth found that dystrophic muscle degenerates much faster than normal muscle. Using this method, bits of muscle placed in

chambers isolated from the rest of the animal body are able to get nutritional fluids through the wall of the chamber.

Still another discovery of biochemical abnormality present in neuromuscular disease provides a promising lead to effective therapy. McArdle's disease is the only hereditary muscle affliction in which the metabolic abnormality has been discovered. The deficiency is phosphorylase. Because of a lack of the enzyme which helps alter muscle glycogen to energy-forming glucose for muscle contraction, normal glycogen accumulates. Electron microscopic studies indicate that the glycogen accumulates under the delicate elastic sheath (sarcolemma) covering muscle fibers, thus compressing the fibers and contributing to muscle weakness.

Myasthenia gravis, the second most serious and life-threatening neuromuscular disease in numbers of patients in the United States, is a muscular disorder with a fluctuating course and severe effects. Certain forms of the disease can be controlled with drug therapy. The disorder most frequently and seriously afflicts young women in the childbearing years. Women with myasthenia gravis may bear children with neonatal myasthenia, which can be fatal. Institute studies of the relationship between a mother's myasthenia and that of her offspring have indicated that a circulating factor may pass through the placental wall from mother to fetus.

Recent Institute research in myasthenia gravis suggests that an autoimmune process may be intimately associated with pathogenesis of the disease. Antibodies with an affinity for certain parts of muscle and thymus have been demonstrated in the circulating blood of patients with myasthenia. Such antibodies have been most frequently demonstrated at highest levels in the blood sera of patients with disease of recent onset and in those patients with severely progressive illness. However, studies this year suggest that these antibodies are not selectively related to the neuromuscular junction. Their effects are non-specific, and a cause and effect relationship to myasthenia is now doubted.

Therapeutic agents for myasthenia gravis are being constantly investigated. Institute-supported research has decisively rejected the use of extracts from the adrenal cortex (ACTH) for treating myasthenia gravis.

Institute clinicians are developing the protocol for evaluating another suggested form of therapy in myasthenia gravis--removal of the thymus gland. By removing the gland at an earlier stage of the disease than previously, physicians predict greater success of patients in recovering from the disease.

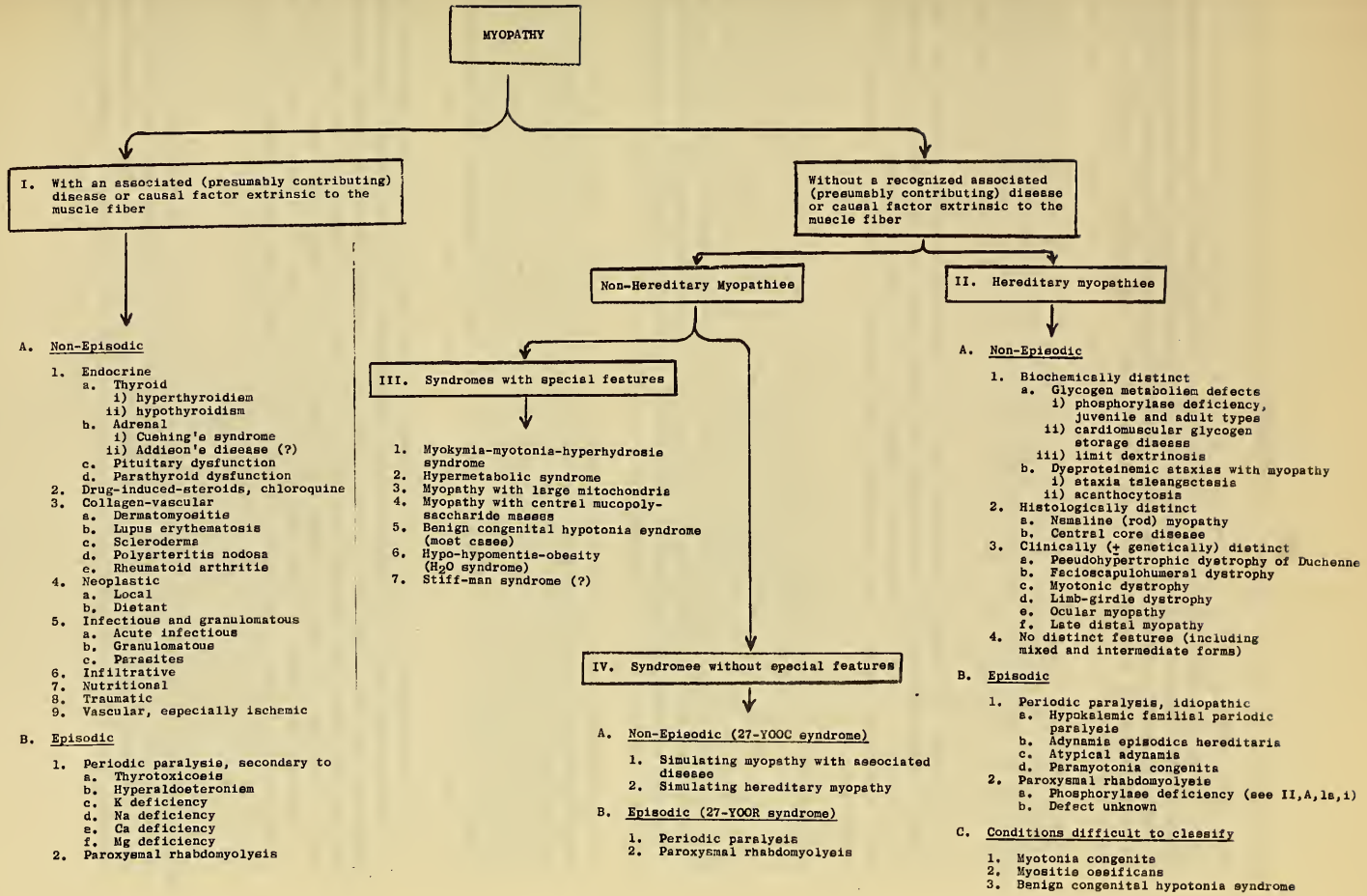
Patients at the Institute with inflammatory myopathies have responded well to treatment with corticosteroids in combination with a vigorous program of physiotherapy and careful clinical management. Studies in the basic nature of the inflammatory myopathies will continue, along with this empirical approach to therapy.

Institute plans for the study of neuromuscular diseases are based on the prospect of controlling the debilitating process in muscle as quickly as possible. To that end, the Institute seeks discovery of the fundamental

defects involved in the structure, chemistry, and function of the affected tissue through its research grant programs and intramural studies.

Complete understanding of the neuromuscular disease processes will be based upon correlations of biochemical and structural defects with disorders in nerve and muscle physiology. Accumulated evidence indicates that both areas provide fruitful answers to the many questions that arise in the course of studying neuromuscular diseases.

In the neuromuscular field, the Bethesda laboratories of the Institute will pursue six major techniques in the coming year: enzyme histochemistry, biochemistry, tissue culture, electron microscopy, autoradiography, and immunology. In addition to the laboratory research approach, scientists continue to develop clinical research techniques. By seeking diagnostic leads, Institute physicians are more effectively able to apply therapeutic approaches.


MYOPATHY
I. With an associated (presumably contributing) disease or causal factor extrinsic to the muscle fiber
Without a recognized associated (presumably contributing) disease or causal factor extrinsic to the muscle fiber
Non-Hereditary Myopathies
II. Hereditary myopathies
III. Syndromes with special features
IV. Syndromes without special features
A. Non-Episodic
1. Endocrine
a. Thyroid
i) hyperthyroidism
ii) hypothyroidism
b. Adrenal
i) Cushing's syndrome
ii) Addison's disease (?)
c. Pituitary dysfunction
d. Parathyroid dysfunction
2. Drug-induced-steroids, chloroquine
3. Collagen-vascular
a. Dermatomyositis
b. Lupus erythematosis
c. Scleroderma
d. Polyarteritis nodosa
e. Rheumatoid arthritis
4. Neoplastic
a. Local
b. Distant
5. Infectious and granulomatous
a. Acute infectious
b. Granulomatous
c. Parasites
6. Infiltrative
7. Nutritional
8. Traumatic
9. Vascular, especially ischemic
B. Episodic
1. Periodic paralysis, secondary to
a. Thyrotoxicosis
b. Hyperaldosteronism
c. K deficiency
d. Na deficiency
e. Ca deficiency
f. Mg deficiency
2. Paroxysmal rhabdomyolysis
1. Myokymia-myotonia-hyperhydrosis syndrome
2. Hypermetabolic syndrome
3. Myopathy with large mitochondria
4. Myopathy with central mucopoly-saccharide masses
5. Benign congenital hypotonia syndrome (most cases)
6. Hypo-hypomentia-obesity (H_2O syndrome)
7. Stiff-man syndrome (?)
A. Non-Episodic (27-YOOC syndrome)
1. Simulating myopathy with associated disease
2. Simulating hereditary myopathy
B. Episodic (27-YOOR syndrome)
1. Periodic paralysis
2. Paroxysmal rhabdomyolysis
A. Non-Episodic
1. Biochemically distinct
a. Glycogen metabolism defects
i) phosphorylase deficiency, juvenile and adult types
ii) cardiomuscular glycogen storage disease
iii) limit dextrinosis
b. Dysproteinemic ataxias with myopathy
i) ataxia telangectasis
ii) acanthocytosis
2. Histologically distinct
a. Nemaline (rod) myopathy
b. Central core disease
3. Clinically (+ genetically) distinct
a. Pseudohypertrophic dystrophy of Duchenne
b. Fascioscapulohumeral dystrophy
c. Myotonic dystrophy
d. Limb-girdle dystrophy
e. Ocular myopathy
f. Late distal myopathy
4. No distinct features (including mixed and intermediate forms)
B. Episodic
1. Periodic paralysis, idiopathic
a. Hypokalemic familial periodic paralysis
b. Adynamia episodica hereditaria
c. Atypical adynamia
d. Paramyotonia congenita
2. Paroxysmal rhabdomyolysis
a. Phosphorylase deficiency (see II,A,1a,i)
b. Defect unknown
C. Conditions difficult to classify
1. Myotonia congenita
2. Myositis ossificans
3. Benign congenital hypotonia syndrome

EPILEPSY

The term "epilepsy" is applied to a group of diseases characterized by the occurrence of repeated episodes of sudden overactivity of the nervous system. These episodes are commonly associated with convulsions, or occasionally with lapses of consciousness or episodic abnormalities of consciousness, thought, or behavior. It is believed that almost 2 million Americans are affected by this disorder.

At present in the majority of cases, seizures may be prevented or reduced by medicines. In a limited number of instances, cure of epilepsy can be accomplished by control of some underlying disease, or by the surgical removal of an area of diseased brain tissue. However, to find the cause and to prevent or completely control the seizures, the National Institute of Neurological Diseases and Blindness is pursuing a broad research program. Its goals are to determine the basic brain mechanisms underlying the seizure, to discover the disease processes which are responsible, to learn the mode of action of anticonvulsant drugs, and to develop and evaluate current and new methods of therapy.

The essential feature of the epileptic attack is the occurrence within the nervous system of a sudden, intense, repeated, electrical discharge of groups of nerve cells. An understanding of this process will require an intimate knowledge of the most fundamental processes of nerve activity and interactions. The investigation of this process represents a large segment of the Institute's "non-categorical" research activities. To provide manpower for this effort, there have been included within the training program $533,000 for training programs in neurophysiology, and 33 traineeships in this field.

The development of more sensitive amplifiers has made possible the better understanding of the essential nature of the electrical nerve discharge, and the recording of the electrical changes which accompany nerve activity. To make this information more meaningful, the Institute is supporting a pioneer computer EEG laboratory in Paris, and has aided in the development of a computer center for neurological research at the University of California at Los Angeles.

The microelectrode, capable of piercing and recording electrical activity from a single nerve cell, is being used to demonstrate the fundamental distortions of electrical activity which occur during the seizure in cells, cell membranes, and at the junctions between nerve cells. This is a powerful tool for evaluating the effects of drugs and chemicals upon these electrical activities. In combination with the EEG, the microelectrode provides insight into the basic functions of brain structures, their interactions, and their response to stimuli. Teaching neurophysiologists this technique is an important segment of the Institute's research training program.

Animal models with artifically induced seizures are being used for the study of the chemical and electrical changes in both the primary and secondary focus. Of particular interest is the fact that the development of such a focus in one area of the brain may lead to the establishment of a secondary focus in another

area possibly due to an electrical bombardment from nerve cells in the primary focus.

Classification of the epilepsies is recognized as an important aid in determining the treatment. Recently seizure classifications have been developed which refer specifically to the particular area of the brain in which the seizure appears to originate. Attempts to classify seizures on the basis of chemical susceptibility of the patient is also under evaluation.

Since the forms of epilepsy vary with the age of the patient, the Institute's Perinatal Research Committee this year sponsored a conference on "Neurological and Electroencephalographic Correlative Studies in Infancy" and the proceedings have now been published.

Also the Institute has established a virus isolation program in search of an infectious agent. Biopsy material from infants seriously afflicted by this disease is being injected into test animals for study.

Another special clinical investigation is being planned using depth and surface electrodes to study the location in which certain seizures originate in the interior of the brain and their relationship and their response to medication.

A practical but highly complex problem that remains to be solved is the determination of the best anticonvulsant or combination of anticonvulsants for a specific patient. The future plans call for large scale and scientifically conducted trials of medicines, with careful classification of cases and evaluation of results, which will provide the necessary information for the practicing physician who uses these powerful agents.

Surgical excision may permanently eliminate seizures in individuals with a clearly defined focus, especially those of the temporal lobe. However, an Institute scientist recently demonstrated that local cooling of an epileptic area of the brain produces alteration in blood vessel permeability and allows the medicines to enter the brain tissue. The value of this technique for the management of previously uncontrolled focal seizures is the subject of another current study of high importance.

An international paired grant involving the University of Wisconsin and the Institute of Brain Research, Zurich, Switzerland, is coordinating special research knowledge and skills of scientists at both centers. They are studying the effect of conditioning upon the stimulus and functioning of the brain, and the field of disturbance of consciousness in persons with epilepsy.

There is a crucial need today for more accurate information regarding the relationship between chemical structure and the action of specific medicines in the body. To aid this need, an Institute program is concentrating on studies relating to the basic mechanisms of the action of anticonvulsant drugs. In spite of structural similarities, studies show important differences between phenobarbital and diphenylhydantoin. Whereas phenobarbital acts to lessen the excitability generally, diphenylhydantoin has little effect on normal sensitivity to seizures, but appears to stabilize affected brain cell membranes and prevent spread of seizure activity.

The Institute is now supporting the reinvestigation of the role of all anti-convulsant drugs in the light of newer knowledge of the electrical and chemical accompaniments of seizures. A special Institute-supported panel has been evaluating methods of testing anticonvulsant agents. Specific problems on testing methods are being clarified prior to commencing the broad testing of anticonvulsants. To aid in this program, the Institute is endeavoring to enlist laboratory and medical specialists at university centers, and researchers from industrial groups.

Investigations in this field are already being undertaken at the Institute-supported Brain Research Institute of the University of California at Los Angeles, in collaboration with its computer center, and at the Neuropharmacology Research Center at the University of Utah, where the mechanism of action of anticonvulsants is a major concern.

Highly important to advancement in the understanding of the overall problem of epilepsy is the Institute support two epilepsy research centers receive. One is located at the University of Washington, where a long-term attack on epilepsy includes the disciplines of neurophysiology, neuroanatomy, neuro-pharmacology, and the behavioral sciences. The other is the Epilepsy Center of the University of Wisconsin which is carrying out studies of drug therapy, together with electroencephalography, and neuropsychological, biochemical, and conditioned reflex research studies.

And to provide a broader base for this effort in the year ahead, the Institute's research program calls for the development of a network of epilepsy study units--clinics within which specific fundamental problems of this disease will be subjected to critical evaluation.

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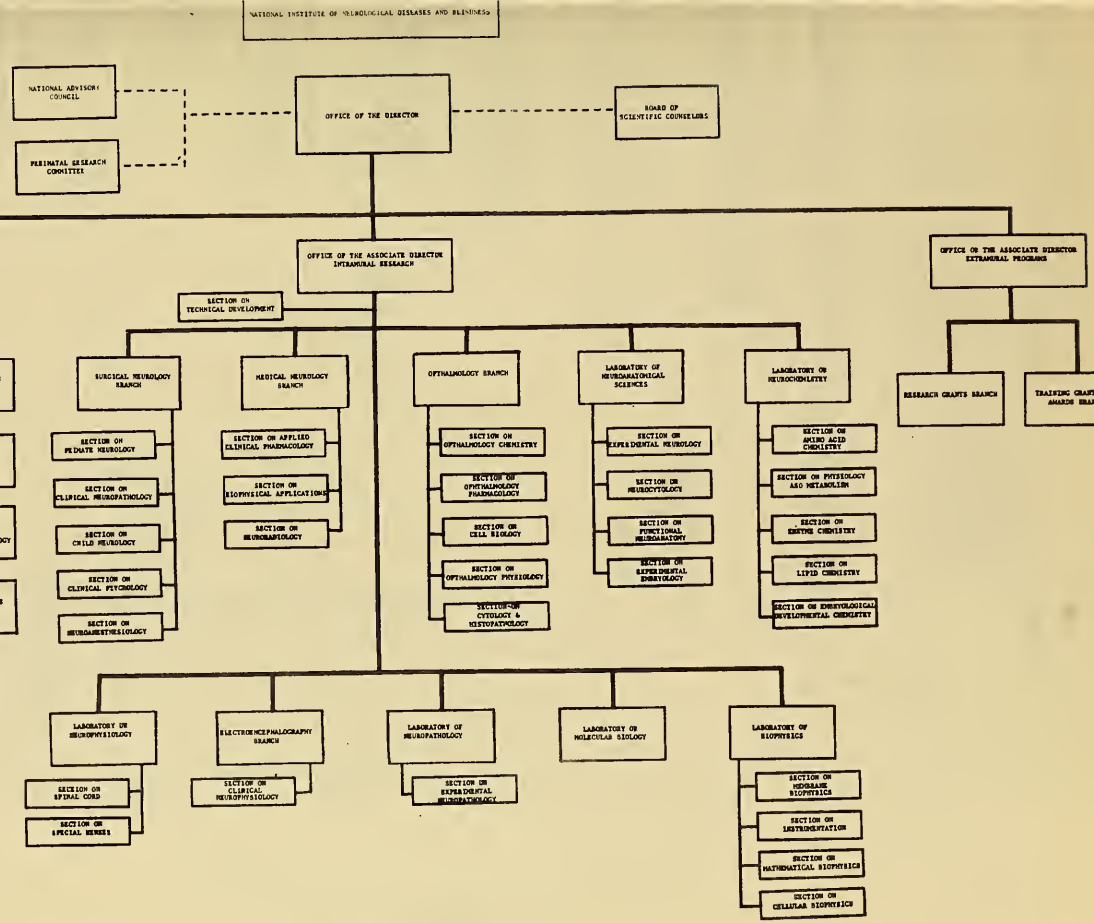
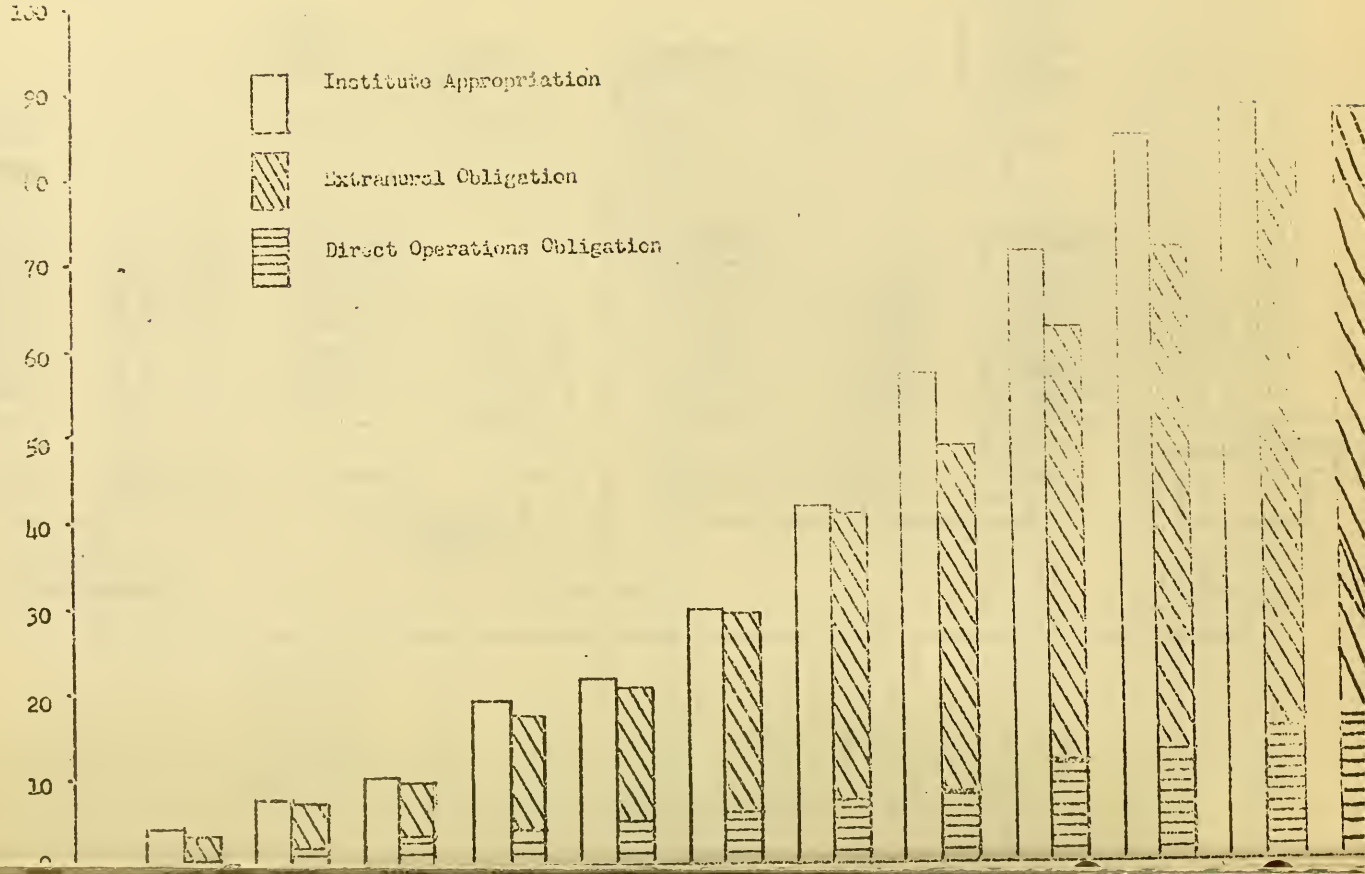
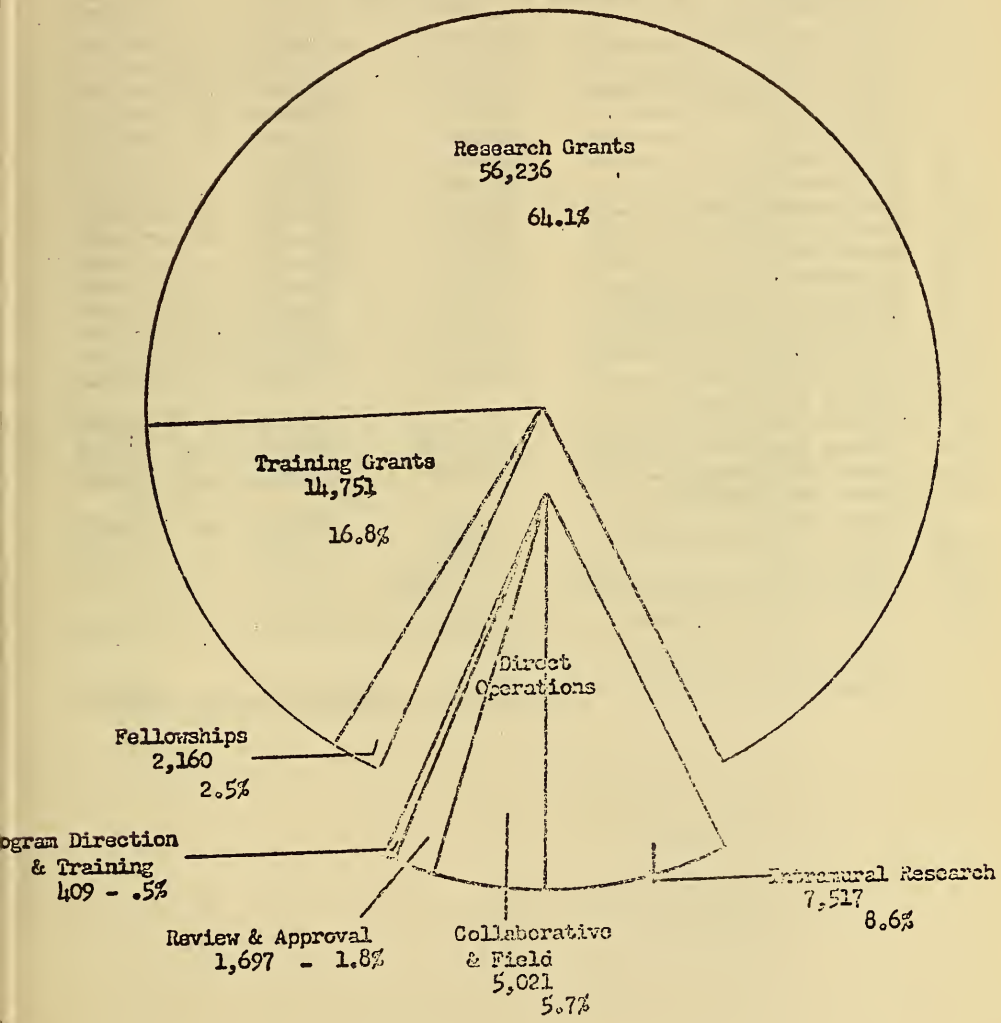
The following charts depict the organizational structure of the Institute and summarize the growth and development of its program--1954-1965.


NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS
NATIONAL ADVISORY COUNCIL
PERINATAL RESEARCH COMMITTEE
OFFICE OF THE DIRECTOR
BOARD OF SCIENTIFIC COUNSELORS
OFFICE OF THE ASSOCIATE DIRECTOR COLLABORATIVE AND FIELD RESEARCH
OFFICE OF THE ASSOCIATE DIRECTOR INTRAMURAL RESEARCH
OFFICE OF THE ASSOCIATE DIRECTOR EXTRAMURAL PROGRAMS
SECTION ON TECHNICAL DEVELOPMENT
PERINATAL RESEARCH BRANCH
ECTION ON EPIDEMIOLOGY & GENETICS
SECTION ON OBSTETRICS
SECTION ON PEDIATRIC NEUROLOGY
SECTION ON BEHAVIORAL SCIENCES
SECTION ON SYSTEMS DESIGN AND PROCEDURES
SECTION ON PROJECT SERVICES
SECTION ON PATHOLOGY
SECTION ON INFECTIOUS DISEASES
SECTION ON STATISTICAL ANALYSIS
EPIDEMIOLOGY BRANCH
BIOMETRICS BRANCH
LABORATORY OF PERINATAL PHYSIOLOGY
SPECIAL PROJECTS BRANCH
SURGICAL NEUROLOGY BRANCH
SECTION ON PEDIATRIC NEUROLOGY
SECTION ON CLINICAL NEUROPATHOLOGY
SECTION ON CHILD NEUROLOGY
SECTION ON CLINICAL PSYCHOLOGY
SECTION ON NEUROANESTHESIOLOGY
MEDICAL NEUROLOGY BRANCH
SECTION ON APPLIED CLINICAL PHARMACOLOGY
SECTION ON BIOPHYSICAL APPLICATIONS
SECTION ON NEUROBIOLOGY
OPTHALMOLOGY BRANCH
SECTION ON OPTHALMOLOGY CHEMISTRY
SECTION ON OPHTHALMOLOGY PHARMACOLOGY
SECTION ON CELL BIOLOGY
SECTION ON OPTHALMOLOGY PHYSIOLOGY
SECTION ON CYTOLOGY & HISTOPATHOLOGY
LABORATORY OF NEUROANATOMICAL SCIENCES
SECTION ON EXPERIMENTAL NEUROLOGY
SECTION ON NEUROCYTOLOGY
SECTION ON FUNCTIONAL NEUROANATOMY
SECTION ON EXPERIMENTAL EMBRYOLOGY
LABORATORY OF NEUROCHEMISTRY
SECTION ON AMINO ACID CHEMISTRY
SECTION ON PHYSIOLOGY AND METABOLISM
SECTION ON ENZYME CHEMISTRY
SECTION ON LIPID CHEMISTRY
SECTION ON EMBRYOLOGICAL DEVELOPMENTAL CHEMISTRY
RESEARCH GRANTS BRANCH
TRAINING GRANTS AWARDS BRANCH
LABORATORY OF NEUROPHYSIOLOGY
SECTION ON SPINAL CORD
SECTION ON SPECIAL SENSES
ELECTRONENCEPHALOGRAPHY BRANCH
SECTION ON CLINICAL NEUROPHYSIOLOGY
LABORATORY OF NEUROPATHOLOGY
SECTION ON EXPERIMENTAL NEUROPATHOLOGY
LABORATORY OF MOLECULAR BIOLOGY
LABORATORY OF BIOPHYSICS
SECTION ON MEMBRANE BIOPHYSICS
SECTION ON INSTRUMENTATION
SECTION ON MATHEMATICAL BIOPHYSICS
SECTION ON CELLULAR BIOPHYSICS

NINDB Appropriation vs Obligation
History



1965 Apportionment Pie Chart
by Activity
(in thousands)
Total--87,821



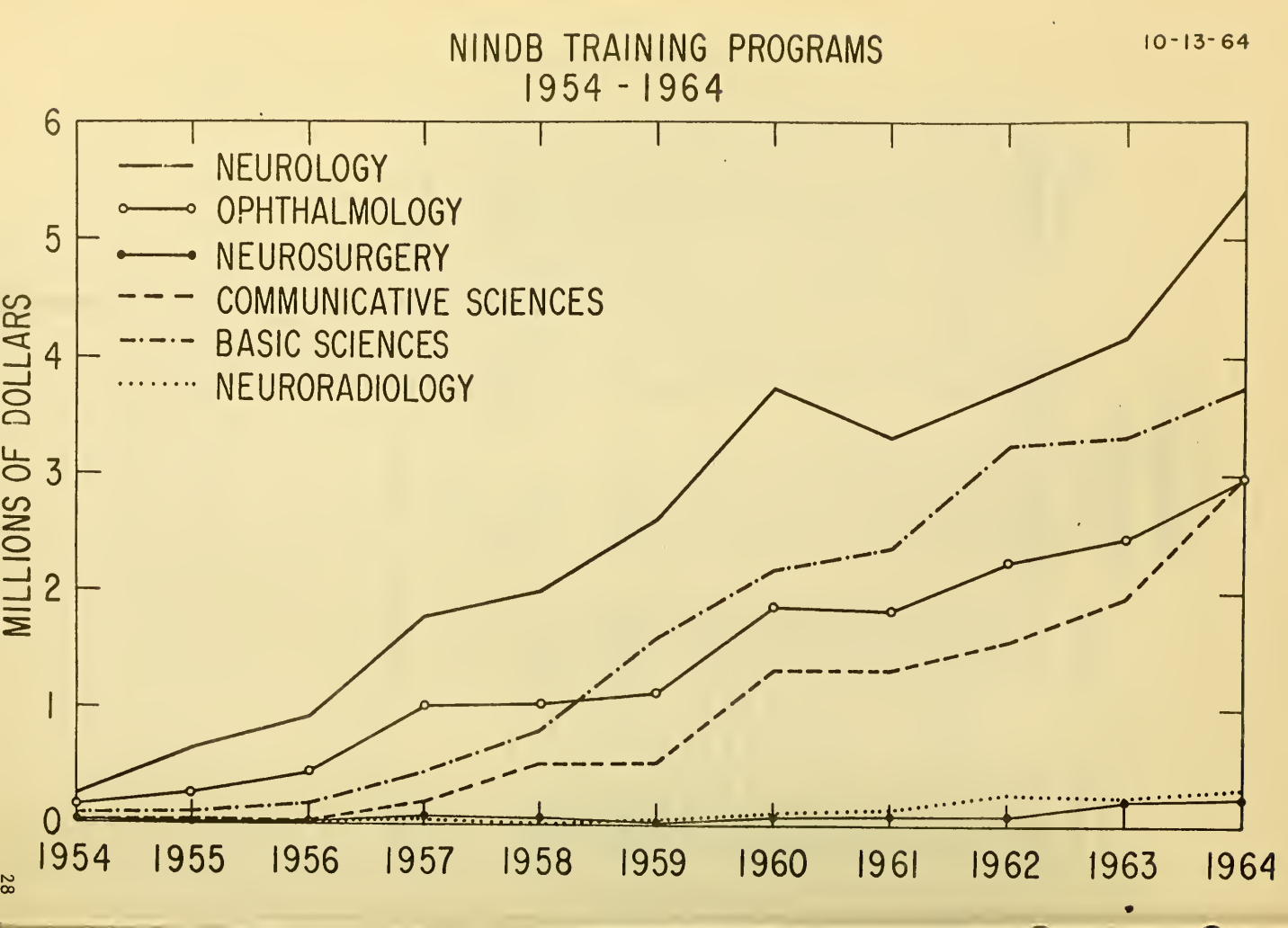
TRAINING GRANTS
Program Analysis

	Fields	1965 Estimate	1966 Estimate	Increase or Decrease
1.	Neurology	$4,638,000	$4,901,000	+$263,000
2.	Ophthalmology	2,040,000	2,157,000	+117,000
3.	Otolaryngology	1,835,000	1,940,000	+105,000
4.	Auditory Physiology	50,000	52,000	+2,000
5.	Communications Disorders	255,000	270,000	+15,000
6.	Medical Audiology	262,000	278,000	+16,000
7.	Neuranatomy	181,000	191,000	+10,000
8.	Neurochemistry	214,000	226,000	+12,000
9.	Neuropathology	633,000	670,000	+37,000
10.	Neuropharmacology	203,000	215,000	+12,000
11.	Neurophysiology	533,000	564,000	+31,000
12.	Neuroradiology	147,000	155,000	+8,000
13.	Neurosurgery	175,000	184,000	+9,000
14.	Experimental Neurosurgery	49,000	51,000	+2,000
15.	Ophthalmic Basic Science	112,000	118,000	+6,000
16.	Otolaryngology & Audiology	114,000	120,000	+6,000
17.	Pediatric Neurology	417,000	441,000	+24,000
18.	Medical Neurology and Public Health	41,000	43,000	+2,000
19.	Sensory Physiology	131,000	138,000	+7,000
20.	Speech Pathology	232,000	245,000	+13,000
21.	Speech Pathology and Audiology	85,000	90,000	+5,000
22.	Vision Psychophysiology	9,000	10,000	+1,000
23.	Other	55,000	58,000	+3,000
24.	Scientific evaluation and planning	140,000	140,000	0
	Total Graduate training grants	12,551,000	13,257,000	+706,000

Traineeships

Program Analysis

Fields	1965 Estimate	1966 Estimate	Increase or Decrease
1. Neurology	20	19	-1
2. Ophthalmology	28	27	-1
3. Otolaryngology	3	3	0
4. Audiology Speech Path.	8	8	0
5. Biochemistry	4	4	0
6. Biophysics	9	8	-1
7. Genetics	1	1	0
8. Neuroanatomy	3	3	0
9. Neurochemistry	19	18	-1
10. Neurocytology	5	5	0
11. Neuroendocrinology	2	2	0
12. Neuropathology	22	21	-1
13. Neuropharmology	8	7	-1
14. Neurophysiology	33	32	-1
15. Neuroradiology	13	12	-1
16. Neurosurgery	2	2	0
17. Pediatric Neurology	39	38	-1
18. Sensory Physiology Path.	16	15	-1
19. Speech & Hearing Mechanisms	1	1	0
20. Virology-Immunology	3	3	0
Total	239	229	-10


NINDB TRAINING PROGRAMS
1954 - 1964
10-13-64
NEUROLOGY
OPHTHALMOLOGY
NEUROSURGERY
COMMUNICATIVE SCIENCES
BASIC SCIENCES
NEURORADIOLOGY
MILLIONS OF DOLLARS
0
1
2
3
4
5
6
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964

January 26, 1965

NIMDB - Disease Category (Research)--In Thousands

Type of Disorder	1964 Actual						1965 Estimate						1966 President's Budget					
	Extramural		Intramural		Total		Extramural		Intramural		Total		Extramural		Intramural		Total	
	No.	Amount	No.	Amount	No.	Amount	No.	Amount	No.	Amount	No.	Amount	No.	Amount	No.	Amount	No.	Amount
Chronic Neurol. of Childhood	136	$10,063	82	$3,789	218	$13,852	139	$10,722	85	$3,922	224	$14,644	139	$11,121	88	$4,083	227	$15,204
Chronic Neurol. Dis. of Aging	72	2,106	12	725	84	2,831	73	2,232	13	751	86	2,983	73	2,315	13	781	86	3,096
Cerebrovascular Disorders	80	2,668	13	271	93	2,939	81	2,833	13	278	94	3,111	81	2,939	14	290	95	3,229
Epilepsy & Related Paroxysmal Dis.	85	2,153	16	1,006	101	3,159	86	2,278	17	1,041	103	3,319	86	2,362	17	1,084	103	3,446
Sclerosing Disorders	83	2,059	35	435	118	2,494	85	2,219	36	448	121	2,667	85	2,301	38	466	123	2,767
Muscular & Neuromus. Disorders	118	2,621	24	952	142	3,573	120	2,785	25	981	145	3,766	120	2,889	26	1,021	146	3,910
Infectious Neurol. Disorders	16	370	15	415	31	785	16	378	15	424	31	802	16	392	16	441	32	833
Tumors of the Nervous System & Trauma	66	1,357	17	930	83	2,287	67	1,469	18	968	85	2,437	67	1,524	18	1,008	85	2,532
Other Neurological Disorders	321	7,161	35	724	356	7,885	325	7,616	36	751	361	8,367	326	7,900	38	781	364	8,681
Disorders of Vision	315	8,338	41	1,807	356	10,145	320	8,871	43	1,876	363	10,747	321	9,202	44	1,953	365	11,155
Disorders of Hearing & Equilibrium	139	4,072	4	275	143	4,347	141	4,321	4	291	145	4,612	142	4,482	4	302	146	4,784
Disorders of Speech & Other Higher CNS Functions	34	796	2	148	36	944	34	831	2	157	36	988	34	862	2	164	36	1,026
Other Sensory Disorders	84	1,638	8	131	92	1,769	85	1,730	8	133	93	1,863	85	1,794	8	139	93	1,933
Non-categorical	25	1,404	6	85	31	1,489	30	1,468	6	85	36	1,553	30	1,523	6	88	36	1,611
Subtotal	1,574	46,806	310	11,693	1,884	58,499	1,602	49,753	321	12,106	1,923	61,859	1,605	51,606	332	12,601	1,937	64,207
General research support grants		4,030				4,030		5,238				5,238		5,219				5,219
Categorical clinical centers		1,200				1,200		1,200				1,200		2,000				2,000
Scientific evaluation and planning		65				65		45				45		65				65
Total	1,574	52,101	310	11,693	1,884	63,794	1,602	56,236	321	12,106	1,923	68,342	1,605	58,890	332	12,601	1,937	71,491

Program Analysis: The Office of Program Analysis has started the development of a scientific information system: (1) to provide facilities for the analysis of special problems and questions which arise relating to the mission of the NINDB; (2) to describe areas of knowledge, and (3) to define problems and gap areas. The input of this system will include Institute research and pertinent research by other Government agencies and non-government organizations. This information will be used to develop both short-and long-range Institute plans.

Effective program analysis requires the talents of more highly trained specialists than the Institute expects to assemble on its staff in Bethesda, so several other analysis techniques are being developed and tested. These include the staff review, the use of advisory committees of the staff, NANDB Council Subcommittees, the scientific advisory councils of voluntary agencies, special topic workshops, visiting scientist reviews, and specialized information centers. The staff reviews, committees and workshops are recognized techniques, but the specialized information centers program represents a new concept and its development is reported in detail.

The specialized information center program of the NINDB was begun in 1963. It was organized to handle increasing amounts of published scientific material by providing a distillation and repackaging of information in specific categorical areas to be more useful to physicians and scientists. These "information centers" are two part structures: acting on one hand as a specialized library to collect, identify, store, and retrieve the various documents concerned with the problem at hand and on the other hand to evaluate, distill, and otherwise utilize the material gathered by the library activities. There are to be two kinds of information centers: (1) a full information center having both parts and (2) a modified information center which has only the evaluative function and draws from another full center for library services.

The full information centers will be placed in great universities where there is a strong biomedical library backed up by a large, general library. These centers will have computer capabilities and personnel in the university to handle library materials by computer methods. There shall be a strong ongoing research program in the categorical area of the center.

The modified information center will be located in centers where there is a strong, ongoing research program in some categorical area of importance to the NINDB, even if there is only a modest library. In these modified information centers, the activities will be concerned with the handling of the information, while the library functions will be carried out at some other full information center.

The information centers are to operate as an interdependent network closely related to the National Library of Medicine. All systems of the Library of Medicine such as the MeSH headings of MEDLARS, and the MEDLARS system, etc., will be the overriding controls for the specialized information centers. This will insure compatibility not only with each other but with the National

Library of Medicine and the many other special information systems which are currently being developed.

There are three centers operating at the present time. The Parkinson's Disease Information Center at Columbia University is the only full center in operation. The other two are feasibility studies. One is for information on higher brain function at UCLA and the other on speech and human communication at Johns Hopkins University. Despite the fact that two are still in the feasibility stage, there has been considerable cooperative development work of the information network. This has been chiefly in the area of the library services while the development of the handling of the scientific information is being worked out separately.

A common glossary control system has been established for developing a thesaurus of terms for the neurological sciences and neurological diseases. This will be computer based and handled by the UCLA information center. Each term is entered with definition, scope notes and authority for the term as well as the center which introduced it. Terms of many languages will be introduced. A special subcommittee composed of members of all the centers will meet to choose the most favorable term and work out problems relating to synonyms.

Common indexing philosophies are being discussed and a uniform system will be adopted and developed. Uniform machine methods will also be adopted.

The translation and examination of foreign language journals has been divided by mutual agreement, UCLA taking the Japanese and oriental languages, Johns Hopkins the Spanish and South American Journals and Columbia the Eastern European journals. Direct communication and interaction with various other governments, non-government agencies and conducting universities are being developed.

There are many information centers and systems being developed throughout the country by other scientific agencies, other governmental agencies, and by industry. Considerable work is being expended in identifying these and establishing some kind of interchange relationship whenever possible.

Information: The Information Office within the Office of the Director discharged the bulk of the Institute's responsibility for planning, developing, and disseminating public information, publications, reports, and special information services generated by (1) members of the Legislative and Executive branches of the Federal Government; (2) the mass media; (3) the health-conscious public; (4) physicians and scientists, and (5) some 30 voluntary health agencies and foundations; and (6) information officers of medical institutions participating in the Institute's grants program.

In fiscal year 1965 the NINDB Information Office served as the Institute's major channel of communication to these professional and lay audiences who, in increasing numbers, inquired about Institute-supported intramural and extramural activities in neurological, sensory, and communicative disorders. As a central resource of facts and figures for the public, and for the medical and scientific community, the information staff responded to a growing demand

for information, with minimal disruption to the research activities of staff scientists and physicians.

Specifically, the Information Office this past year distributed 315,208 publications; answered 1,453 letters of inquiry (exclusive of requests for publications) and approximately 5,100 telephone inquiries; published, or prepared for publication, 23 new or revised brochures, leaflets, and fact sheets; arranged 15 lectures by guest scientists; prepared 8 press releases and announcements; answered 138 press calls from representatives of 75 to 80 scientific and lay publications; cleared 222 Intramural manuscripts; prepared 6 issues of the NINDB Review (a publication for NANDB Council members and voluntary health agencies); prepared 4 issues of the Collaborative Reporter for participating institutions of the Collaborative Project; developed one new exhibit, in cooperation with the Deafness Research Foundation, and the American Academy of Ophthalmology and Otolaryngology; supervised production and completion of a new NINDB program exhibit; manned NINDB exhibits at 10 meetings; cooperated in producing or supplying information for one NINDB-supported film; arranged through CDC for 465 showings of Institute-supported films (one, "Exploring the Human Nervous System," a previous prize winner and recipient this year of two new awards); assisted in producing 8 radio and TV shows; provided major editorial services for publication of the report of NINDB mission to Russia to investigate viruses, the proceedings of an Institute-sponsored instrumentation workshop, and the manuscript for a forthcoming book on lower back pain; prepared 32 reports, primarily scientific and technical; wrote 12 speeches and special statements for members of Congress and Government officials; wrote 20 feature articles for the NIH Record.

In addition to this broad effort, the Information Office initiated and executed a variety of public-information and education programs, and special events, required to interpret the Institute's research effort to many audiences.

ANNUAL REPORT
July 1, 1964 to June 30, 1965
Office of the Special Assistant to the Director for Biometry
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

The program of this Office for the 12 months covered by this report has been a diversified one of biometric consultation and counselling on the one hand, and an intensified activity in a long-term research project on the other. The former activities are reviewed hereunder in narrative form; the research program is reviewed in the concluding Individual Project Report.

The research support functions of this office for the past year have included two distinct types of activity in the same general domain: (a) Service functions in an advisory and/or consultant capacity to the collaborative research programs and their principal investigators now supported by grants from the Institute; and (b), further development of a protocol for a proposed collaborative project to investigate the merit of thymectomy in the management of Myasthenia Gravis. These two facets of the field investigations program are reviewed briefly in that order in what follows.

Approximately 10 per cent of the year's time has been given by the Chief of this Office to conferences with staff of extra-mural grant projects in advisory assistance to accomplishment of their defined objectives. The areas of collaborative research, given in the order of their time utilization, have included: Cerebral Accidents, their treatment, and a comparative evaluation of their incidence in different countries; Epilepsy, and the design of investigations for its treatment; Headache, its classification and the development of protocols for testing therapeutic drugs used in their management; and the activities (both general and specific) of the Institute's Advisory Panel on Evaluation of Clinical Therapy, of which group the writer is a full member. The Advisory Panel has been concerned specifically this year with review of a collaborative project in Multiple Sclerosis, as well as with the more general issues involving collaboration in needed areas of neurological research.

The development of a protocol for the evaluation of surgical intervention (thymectomy) in the management of Myasthenia Gravis, as mentioned in the preceding report, is a continuation of activity concerning evaluation of treatments for this disease started elsewhere within this Institute. During the current year this work has involved reconstruction of the original protocol draft, an activity which has engaged approximately 15 per cent of the writer's time. The general design features seem now to be in reasonable form for implementation, but much detail work with suitable medical collaboration needs to be undertaken in the area of definitions and instructions concerning all testing procedures. The organization of an independent Central Office to control procedures and undertake the statistical processing and analysis of data accumulated in this collaborative effort is a crucial administrative issue receiving detailed attention from this office. Reports on the status

of the proposed undertaking are presented by the writer to the semi-annual meetings of the Medical Advisory Board of the Myasthenia Gravis Foundation, which is co-sponsor (with this Institute) of this activity.

Serial No. NDB(OD)-65-1258
1. Office of the Director
2. Special Assistant for Biometry
3. Wiscon Building

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Human Reproduction and the Menstrual Cycle.

Principal Investigator: Alan E. Treloar, Ph.D.

Other Investigators: Borghild G. Behn, Ph.D.

Cooperating Units: University of Minnesota
a) Biostatistics Department, Drs. J. E. Bearman and B. W. Brown, Jr.
b) Health Service, Dr. D. W. Cowan

Man Years, Total: 4.7
Professional: 1.7
Other: 3.0

Project Description.

Objectives: a) To define quantitatively for a large sample of women the pattern of temporal variation in the menstrual cycle throughout the span from menarche to menopause.

b) To secure accurate records of the interruptions of menstrual function through pregnancy, and through current recording of all dates to define reliably the gestational interval from onset of last menstrual period to delivery of the product of pregnancy.

c) To study the effects on menstrual cyclicity of the use of pregnancy control medications in the form of hormonal steroids (the various "birth control pills").

Methods Employed: Enrollment of young women attending the University of Minnesota in a research plan to maintain accurate records of all menstrual flow periods was commenced by the chief of this office in 1934. Over 500 collaborators secured in the initial approach successfully launched the program, and for the following four years like numbers of new collaborators were secured each year. Mail contact has been maintained as far as possible with all collaborators each year, completed records and medical reports being collected as new calendar cards were issued. Later, daughters of these collaborators were invited to join the study as they reached menarche. Approximately five years ago, collaborators experiencing menopause and hysterectomy reached a level suffi-

cient to justify an analysis of the accumulated records being undertaken with adequate coverage (in numbers of persons) through the years of menstrual life. Although at that time the writer joined the NIH staff, the project was maintained in most of its operations at the University of Minnesota, where it is very active still.

A new group of approximately 1200 university women has been enrolled in this study through the past few years to enable a comparison for the same sort of sample to be made after a quarter-century time lapse. Accumulation of records from this new group will be adequate in relatively few years to enable a dependable judgment to be reached through age-specific analysis.

Preparation of the data from the original collaborators and their daughters for analysis by computer operations was started in 1961 at the University. Computer runs with printed output from tested programs were achieved in the third year of these operations. The specific period covered by this present report has been one of assembly of the products of these operations into narrative presentation of the accumulated results for all calendar years from the program inception through 1961. A draft of the first report on age-specific menstrual cycles has been completed. It was made available to members of the Population Association of America as one basis for a seminar held on 24 April last at their annual meeting. Another report is in process of assembly dealing with the gestational intervals for the 2062 live births to the collaborators during this period.

Major Findings: With approximately 26,000 person-years of experience available for these analyses, a composite picture of the age-specific of menstrual intervals covering the complete span from menarche to menopause has been developed. Also, an accurate set of records for the gestational interval has been made available in sufficient numbers to be definitive of this variable. Both of these bodies of information are novel in that they either fill voids in the literature or replace fragmentary information by enough cases to generate distributions of great stability.

The menstrual interval study has led to the definition of three age zones in menstrual life. The first eight years of experience must be assembled for all collaborators in terms of menstrual age; this is defined as the menarchial zone. It satisfactorily unites at chronological age 20 (approximately) with the usual age scale with its origin at birth. At age 40 approximately the heterogeneity created by impending menopause for many collaborators necessitates termination of the chronological age zone in favor of a menopausal age scale. This can be generated for any individual only when "absolute" menopause is reach; this is a negative scale proceeding backwards from this point of origin. The menopausal zone so generated is approximately 10 years in span, or about two years wider than the menarchial age zone. Frequency contours have been secured for this joint distribution of menstrual interval by age (in the three zones, and each by single years). These contours, which proceed by deciles from 10 to 90 per cent, and by percentiles from one through five and from 95 through 99 per cent, are supplemented also by the quartile values (25 and 75 per cent). Except for the extreme boundaries (one, two, 98 and 99 percentiles) these empirical contours are

so remarkably stable that there is no point to attempting further smoothing by mathematical processes of graduation.

The following conclusions have been reached from these analyses:

Menstrual Intervals produced in the commonly stated pattern of "regular, every 28 days" are so rare that this pattern may be dismissed as a fiction unless only very brief periods of record are used. After menarche, which usually (but not always) initiates very great irregularity in menstrual interval, there is a progressive decrease in this irregularity through the menarchial zone (8 years) before the stability of middle life and the usual pregnancy years is reached. The median menstrual interval per person tends to decrease slightly — by about 2 days — in a straight line manner from age 20 to just before the menopausal experience is initiated around 40 years of age. Through this same period there is a more marked progression toward greater regularity in menstrual interval. The standard deviation by chronological age changes on the average from 4 days (approximately) at age 20 to 3 days (approximately) at age 40. This decrease in irregularity is over 20 per cent. For most women, birth control by following the rhythm method is not practicable except perhaps in the last half of the "thirties". The menopausal zone of approximately nine years is remarkably like a mirror image of the menarchial in its changing irregularity.

Short menstrual intervals tend to increase in number with the long intervals in both the menarchial (initial) and menopausal (terminal) zones of menstrual life. Because of this the median interval changes slowly with time in these zones as opposed to the variability.

When individual histories are traced, their most impressive feature is the individuality of women with respect to menstrual interval. Each person has her own characteristics of mean interval and its change with time, and of stability (or lack of it) in variability of these intervals between menstrual onsets. There is no assurance that the pattern of menstrual intervals as observed even over several years will not change either suddenly or with considerable acceleration. Forecasting the day of next ovulation, if it is to be made without risk of failure, must be based on more than past menstrual history; it must be supplemented by such observations as physical and/or psychological symptoms plus daily basal body temperature determinations.

There is no detectable correlation between age at menarche and age at menopause. This negates a traditional viewpoint in the gynecological literature. Age at menarche as recalled a few years after this event varied for the original collaborators (born approximately 1895 to 1920) from age 9 to 19, with a mean at 12.7 years. Mean age at menarche thus given decreased slowly and progressively through these years over a span from 13.8 to 12.3 years; this change is statistically significant. Comparison with those above of the currently recorded age at menarche for the daughters is awaited with interest; the span of years covered is already approaching the same range of roughly 20 years. Age at menopause for the original collaborators is also being watched closely; for 120 cases at present available it ranges over 21 years, from 36 to 56. The average

presently stands at approximately 48½ years, but this is expected to increase as the distribution becomes enhanced by a better balance of the more advanced ages.

The definitive outcomes of the massive study of menstrual intervals is matched on a very different score by the challenging results of the gestational intervals. The essential agreement between the results from within the menstrual history program with those obtained from a number of totally different sources for the white race reject the view held by the overwhelming majority of the obstetrical profession that "you can't believe what a pregnant woman tells you about her last menstrual period." Our currently recorded data agree splendidly with hospital records using recalled LMP dates for white. The evidence therefore arises from very divergent sources that the traditional gestational interval (LMP to birth) varies in fact over a range which may be given as approximately from 30 to 46 weeks. Evidence from the menstrual history study makes it reasonable to explain this wide range in terms of two propositions:

(1) Cyclic bleeding in step with previous menstrual history persists for an indeterminable proportion of women for one or more "apparent menses", this generating the short gestational intervals.

(2) Abortions, mostly unknown, are followed by viable pregnancies without an intervening menstruation in enough cases to account fully perhaps for the long gestational intervals.

Using data supplied by the Perinatal Research Branch, there is clear indication that whereas a diversity of sources for data on Caucasian races show a uniformity in variation pattern of gestational intervals, the American Negro race diverges significantly toward shorter gestational intervals. The difference of approximately six days on the average is supplemented by a different pattern of frequency distribution for this variable. The latter point suggests that perhaps post-pregnancy cyclic bleeding for the Negro is more common than for Caucasians.

Significance to Bio-Medical Research. The findings concerning menstrual cyclicity and gestational intervals as set forth above are deeply challenging in areas where traditional concepts have hampered progress. The facts about to be published, if accepted, must have a weighty impact on each of two distinct frontiers of research. The problems of birth control will be recognized to require extensive revision of the claims for adequacy of the rhythm method. The hormonal control systems for the menstrual function as evidence of failure of pregnancy will need to be reviewed for a shift from a deterministic basis to a probabilistic basis. This would be a type of change commonly experienced in the sciences as maturity is progressively achieved.

Proposed Course of Project. It is anticipated that this research program will continue under its present direction. Hopefully this will be achieved with the continued cooperation of the University of Minnesota if its administrative provisions will permit. However, the program is not dependent on that cooperation, but rather on continuance of its present direction and professional staffing in

a setting advantageous to its fullest development.

The study of a similar population selected 25 years after the original one will give valuable evidence on any apparent change in menarche and menstruation periodicity through that interval. It is planned that extensions of the study should include new population groups of the following types:

a) The American Negro.

b) The Eskimo as a race in a frigid climate not too far removed from their earlier native state.

c) The Carolinian and Mariana Island natives (or a similar group) contrasting with the Eskimo at least in being in a tropical setting.

d) A southern hemisphere Caucasian group such as the Australian, which has much in common with the United States whites but contrasts in the hemispheric environment.

e) National or racial groups from other sectors of the globe which may choose to add menstrual history study to other related activities of other on-going programs.

A unique opportunity presents itself for study of the effect of "birth control pills" on the course of menstrual cyclicity in the younger members of the original collaborator group. Provision for accumulation of data bearing on this issue has already been made and analyses of results can be initiated in the near future. It is intended that this will be accomplished as part of a plan now being developed to code all available data (instead of intervals only, through year 1961) comprehensively for computer analysis. Data collected through the years 1962-3-4 already in hand await coding in accord with the broadened objectives. Another eight to ten years of intensive analytical work supplementing expanded data collection is envisaged before this study will perhaps be drawn to a close.

Part B included No

ANNUAL REPORT

July 1, 1964 through June 30, 1965

DIRECT TRAINING

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS
NATIONAL INSTITUTES OF HEALTH

Funds under this activity provide for the support of the Institute's training programs for professional, technical, administrative, and clerical personnel, including job-related training at both government and non-government facilities.

The Institute is currently supporting the training of an oto-laryngologist at John Hopkins University, a neurologist at Mt. Sinai Hospital, and an epidemiologist at the Communicable Disease Center in Atlanta, Georgia.

In addition, two Commissioned Officers from the Intramural Research programs are receiving neurological and neurosurgical training at the Barnes Hospital, Washington University, St. Louis, Missouri and at the State University of New York, Syracuse, New York.

Short term courses are also supported as funds are available.

Recently a committee to advise the Director on Training Outside the Service has been created.

ANNUAL REPORT
Fiscal Year 1965
Extramural Programs
National Institute of Neurological Diseases and Blindness
National Institutes of Health

I. Purpose:

The Extramural Programs of the NINDB have as their primary objective the identification of research areas of national need in the neurological, neurosensory, and communicative fields and the stimulation and support of selected biomedical research and training in those areas. The research grant, training grant, and fellowship programs provide funds on a competitive basis for the manpower, resources and facilities necessary to meet this objective.

II. Programs:

A. Research Grants

The research grant program including regular research projects and clinical center-program projects provides funds for the initiation and support of research on basic and clinical problems related to the nervous system and neuromuscular systems; the special senses including vision, hearing, smell, touch, and pain; and communications including speech and language. In the neurological area special emphasis was given to the problems of epilepsy, multiple sclerosis, cerebrovascular disease, muscular dystrophy, cerebral palsy, and other neurological and neuromuscular disorders; in the area of vision, special attention was given to the problems of glaucoma, cataract, uveitis, infections and congenital abnormalities; in the area of hearing, special emphasis was given to otosclerosis, nerve deafness, middle ear and inner ear infections, and congenital disorders; and, in communicative disorders, special attention was given to aphasia, problems of voice production and modulation, speech development, and central nervous system information storage and organization. (See exhibits 1 a and 1 b.)

Through the research grant program the NINDB awarded in FY 65 a total of 1,615 research grants totaling $50.9 million; of these, approximately 260 grants totaling $7.0 million were awarded for needed new research undertakings, and $44 million was provided to continue or supplement 1,355 successful on-going research projects.

Also through its research grants program, NINDB supported a total of 24 clinical center-program projects at a level of $4.96 million; 16 in the area of neurological disorders and 8 in the area of sensory and perceptual disorders. (See exhibit 2.)

B. Training Grants

The training grant program provides funds on a competitive basis for

the establishment, improvement, and support of training in the disciplinary areas related to the neurological, neuromuscular, sensory or communicative disorders. The primary objective of the training grant program is to train needed additional teacher-investigators, scientist-physicians, and community health and public health personnel. In fiscal year 1965 the NINDB awarded 248 graduate training grants totaling $12.4 million; 13 grants totaling $842,000 were awarded to establish new training programs; and $11.6 million was awarded to continue and supplement 235 active and successful on-going programs. (See exhibit 3.)

In addition, 200 Special Fellowships, providing stipend support for highly specialized research training, were awarded to basic and clinical scientists preparing for careers in research and academic medicine. (See exhibit 4.)

C. Fellowship Program

Through its Fellowship Program, NINDB makes available stipend support in the form of Postdoctoral Fellowships, Career Awards and Career Development Awards. Postdoctoral Fellowships are awarded to scientists and scientist-clinicians desiring specific research training following the receipt of a doctoral award. Career Development Awards provide stipend support for able scientists developing careers in independent research and teaching, and are available primarily for investigators desiring experience and further training in a productive research environment, or for scientists undertaking independent research and in need of further experience to qualify for a senior position. Career Awards provide additional stable positions for experienced, independent investigators in pursuit of productive careers in research and teaching.

In fiscal year 1965, 63 postdoctoral fellowships totaling $438,000 were awarded. Seventy-five research career development awards totaling $1,376,000 and 14 research career awards totaling $327,000 were supported. (See exhibit 5.)

Exhibit 1 a

NUMBERS OF RESEARCH GRANTS SUPPORTED
IN FY 65 AND AMOUNTS ARRANGED BY DISORDER CATEGORY

TYPE OF DISORDER	No.	Amount	% of $
All Disorders	1615	$50,953,000	100
I. NEUROLOGICAL DISORDERS			
A. Chronic Neurological Disorders of Childhood	119	10,516,000	20.6
B. Chronic Neurological Disorders of Aging	65	2,399,000	4.7
C. Cerebrovascular Disorders	79	2,898,000	5.7
D. Epilepsy and Related Paroxysmal Disorders	82	2,330,000	4.6
E. Sclerosing Disorders	91	2,220,000	4.4
F. Muscular & Neuromuscular Disorders	114	3,061,000	6.0
G. Infectious Diseases	16	354,000	0.7
H. Accident and Injury	36	728,000	1.4
I. Tumors of Nervous System	32	659,000	1.3
J. General	367	8,138,000	16.0
ALL NEUROLOGICAL DISORDERS	1001	33,303,000	65.4

		No.	Amount	% of $
ALL DISORDERS		1615	$50,953,000	100
II.	SENSORY & PERCEPTUAL DISORDERS			
	A. Disorders of Vision	329	9,069,000	17.8
	1. Cataract	27	687,000	1.3
	2. Glaucoma	40	1,062,000	2.1
	3. Retinopathy & Neurological Mechanism of Vision	130	3,178,000	6.3
	4. Inflammatory & Parasitic	42	1,484,000	2.9
	5. Metabolic & Degenerative	13	514,000	1.0
	6. Strabismus & Neuromuscular	16	393,000	0.8
	7. Injuries & Other Disorders including Tumors	10	225,000	0.4
	8. General	51	1,526,000	3.0
	B. Disorders of Hearing and Equilibrium	143	4,261,000	8.4
	C. Disorders of Speech & Other Higher CNS Functions	30	979,000	1.9
	D. Disorders of Other Senses	78	1,627,000	3.2
	ALL SENSORY & PERCEPTUAL DISORDERS	580	15,936,000	31.3
III.	NON-CATEGORICAL	34	$ 1,714,000	3.4

Exhibit 1 b

NUMBERS OF RESEARCH GRANTS SUPPORTED
IN FY 65 AND AMOUNTS ARRANGED BY SCIENTIFIC DISCIPLINE

TYPE OF CLASSIFICATION	No.	Amount	% of $
All Classifications	1615	$50,953,000	100
A. NEUROANATOMICAL SCIENCES			
1. Gross neuroanatomy & comparative neurology: Morphological	29	605,000	1.2
2. Gross neuroanatomy & comparative neurology: Experimental	34	573,000	1.1
3. Neurocytology & histology: Morphological	43	1,018,000	2.0
4. Neurocytology & histology: Experimental	58	1,186,000	2.3
5. Embryology, development & regeneration: Morphological	14	263,000	0.5
6. Embryology, development & regeneration: Experimental	33	707,000	1.4
7. Neuropathology: Morphological	13	453,000	0.9
8. Neuropathology: Experimental	37	718,000	1.4
9. Pathology of Sense Organs	46	1,279,000	2.5
ALL NEUROANATOMICAL SCIENCES	307	6,802,000	13.3
B. NEUROPHYSIOLOGICAL & NEUROPHYSICAL SCIENCES			
1. Neurophysiology	155	3,897,000	7.7
2. Neuromuscular Physiology	43	932,000	1.8
3. Biophysics, including Electrophysiology	119	3,303,000	6.5
4. Special Senses	105	3,176,000	6.2
ALL NEUROPHYSIOLOGICAL & NEUROPHYSICAL SCIENCES	422	11,308,000	22.2

TYPE OF CLASSIFICATION	No.	Amount	% of $
All Classifications	1615	$50,953,000	100
C. BIOCHEMICAL SCIENCES			
1. Basic Biochemistry	107	2,451,000	4.8
2. Enzymology	39	1,065,000	2.1
3. Metabolism and Nutrition	99	2,461,000	4.9
4. Pharmacology & Experimental Therapeutics	120	2,516,000	4.9
ALL BIOCHEMICAL SCIENCES	365	8,493,000	16.7
D. SOMATO-NEUROLOGICAL RELATIONSHIPS			
1. Endocrinology	32	807,000	1.6
2. Hematology	37	604,000	1.2
3. Immunology	39	1,043,000	2.0
4. Microbiology	13	325,000	0.6
5. Parasitology	2	18,000	0.1
6. Respiratory	13	272,000	0.5
7. Whole Animal	5	120,000	0.2
8. Genetics	14	446,000	0.9
ALL SOMATO-NEUROLOGICAL RELATIONSHIPS	155	3,635,000	7.1
E. CLINICAL INVESTIGATIONS			
1. Clinical Neurology	121	13,296,000	26.2
2. Clinical Ophthalmology	47	1,734,000	3.4
3. Clinical Otology	35	1,057,000	2.0
4. Clinical Other	13	379,000	0.7
ALL CLINICAL INVESTIGATIONS	216	16,466,000	32.3
F. PSYCHOLOGICAL SCIENCES			
1. Psychology	77	2,046,000	4.0
2. Psychiatry	1	16,000	0.03
ALL PSYCHOLOGICAL SCIENCES	78	2,062,000	4.0

TYPE OF CLASSIFICATION	No.	Amount	% of $
All Classifications	1615	$50,953,000	100
G. OTHER			
1. Epidemiology	6	387,000	0.8
2. Conferences	13	334,000	0.7
3. Publications	6	167,000	0.3
4. Consultations	5	120,000	0.2
5. Instrumentation	39	1,168,000	2.4
6. Other	2	12,000	0.02
ALL OTHER	71	2,188,000	4.4

Exhibit 2

PROGRAM PROJECTS - CLINICAL CENTERS
EXPENDITURES - FY 65
BY DISORDER CATEGORY

	No. of Grants	Amount
NEUROLOGICAL DISORDERS (TOTAL)	*16	3,463,591
General	*5	655,250
Chronic Neurological Disorders of Aging (Parkinsonism)	2	796,762
Cerebrovascular Disorders	3	845,260
Epilepsy and Related Paroxysmal Disorders	2	301,281
	3	699,605
	1	165,433
SENSORY & PERCEPTUAL DISORDERS (TOTAL)	8	1,496,581
General	4	664,931
Disorders of Vision	2	387,418
Disorders of Hearing	2	444,232
OVERALL TOTAL	*24	4,960,172

*Includes one grant, active, but without FY-65 support funds.

Exhibit 3

DISTRIBUTION, BY SCIENTIFIC FIELDS, OF
TRAINING GRANTS SUPPORTED IN FY 65

	No. Grants	Amount Awarded
Neurology	75	$ 4,398,645
Ophthalmology	42	2,313,690
Otolaryngology	41	1,788,965
Auditory Physiology	2	42,653
Communicative Disorders	3	211,819
Medical Audiology	5	222,168
Neuroanatomy	6	246,139
Neurochemistry	3	177,505
Neuropathology	14	495,530
Neuropharmacology	4	168,038
Neurophysiology	13	608,105
Neuroradiology	4	122,331
Neurosurgery	7	170,685
Ophthalmic Basic Science	3	145,226
Otolaryngology and Audiology	1	95,721
Otolaryngologic Pathology	1	164,942
Pediatric Neurology	13	417,071
Preventive Medicine	1	34,959
Sensory Physiology	3	180,025
Speech Pathology	4	183,359
Speech Pathology - Audiology	2	104,522
Vision Psychophysiology	1	8,977
Other	5	100,000
Total	248	$12,401,075

Exhibit 4

DISTRIBUTION, BY SCIENTIFIC FIELDS,
OF SPECIAL FELLOWSHIPS AWARDED IN FY 65

Fields	No.	Amount
Biochemistry	4	$ 25,900
Biophysics	9	116,000
Audiology & Sp. pathology	4	60,900
Immunology	1	8,700
Neurology	25	268,200
Neuroanatomy	12	151,000
Neurochemistry	7	86,100
Neurocytology	2	23,300
Neuroendocrinology	4	49,800
Neuro-ophthalmology	4	41,000
Neuropathology	10	97,600
Neuropharmacology	5	55,000
Neurophysiology	23	290,400
Neuroradiology	15	145,600
Neurosurgery	5	66,900
Ophthalmology	23	238,900
Ophthalmic pathology	10	121,200
Otolaryngology	5	38,800
Pediatric neurology	22	205,400
Sensory physiology (Oto.)	4	43,700
Sensory physiology (Vision)	6	65,600
Total	200	$2,200,000

Exhibit 5

DISTRIBUTION, BY SCIENTIFIC FIELDS, OF FELLOWSHIPS AWARDED IN FY 65

Fields	Research Career Awards		Research Career Development Awards		Postdoctoral Fellowships	
	No.	Amount	No.	Amount	No.	Amount
Audiology	1	25,708	5	71,896	1	7,000
Biochemistry					1	9,420
Biophysics					2	13,766
Genetics					1	7,000
Neuroanatomy	2	33,366	5	82,372	7	41,000
Neurochemistry	1	25,010	4	71,768	7	42,559
Neurocytology			2	29,040	3	24,994
Neuroendocrinology	1	18,490				
Neurology	3	88,833	2	45,404	2	16,000
Neuropathology			2	38,709	5	41,225
Neuropharmacology	2	39,706	14	226,505	4	27,097
Neurophysiology	1	23,997	14	272,715	12	88,666
Neuroradiobiology			1	15,887		
Neurosurgery			1	15,746	2	15,500
Otolaryngology			1	15,628	2	13,500
Otoneurology			2	44,071		
Ophthalmic pathology			6	118,125		
Ophthalmology			2	42,274	5	30,739
Pediatric Neurology			1	25,670	2	13,500
Physiological psychology	1	22,264	3	64,799	3	17,500
Physiological optics			2	36,070		
Sensory physiology						
(Vision)	1	23,794	3	43,970	2	16,066
(Oto.)	1	25,641	2	34,832	1	6,968
Speech & Hearing Mech.			2	34,893		
Virology & Epidemiology			1	16,433		
Totals	14	326,809	75	1,375,854	63	438,000

Table of Organization

Intramural Research

National Institute of Neurological Diseases and Blindness
(Personnel on hand May 1965)

Office of Associate Director:

Acting Associate Director - Karl Frank, Ph.D.
Clinical Director - Maitland Baldwin, M.D., M.S., F.R.C.S.
Administrative Officer - Glenn E. Hammond
Administrative Officer - Nick Kastelan, Jr.
Visiting Scientist - Edward A. Carmichael, M.B., C.B.
Surgeon - Alden W. Dudley, Jr., M.D.
Surgeon - Robert J. Ruben, M.D.
Surgeon - Joseph Crawford, M.D.
Surgeon - William D. Moore, M.D.
S.A. Surgeon - Gerald Gold, M.D.
Photographer - Clarence Kuritzky
Photographer - Wesley Pearson, Jr.
Biologist - W. Frederick K. Seymour
Special Assistant - Nancy F. Nusbaum
Secretary - Mary E. Foley
Secretary - Maxine O. Reynolds
Clerk (Typing) - Doris R. Perry
Travel Clerk - Ida M. Isaacson
Photographer - Herbert G. Alston
Biological Laboratory Technician - George R. Duvall
Clerk-Stenographer - George Ann M. Johnson
Clerk-Stenographer - Virginia L. Gray
Clerk-Stenographer - May I. Ferrari
Biological Laboratory Technician - Adrian P. Loftis
Clerk-Stenographer - Paula B. Pavey
Clerk-Stenographer - Dawn M. Broadhurst
Secretary (Stenographer) - Gertrude C. D'Addio

Section on Technical Development:

Electronic Engineer - Jerome A. Rosenthal
Digital Computer Programmer - William H. Sheriff, Jr.

Medical Neurology Branch:

Chief - W. King Engel, M.D.
Visiting Scientist - Leon A.H. Hogenhuis, M.D.
Surgeon - Arthur H. Wolintz, M.D.
Surgeon - Mahlon R. Barlow, M.D.
Surgeon - Dale McFarlin, M.D.
Surgeon - Jerome Resnick, M.D.
Surgeon - William Collis, M.D.
Surgeon - Donald Mauser, M.D.
Surgeon - Genevieve Drews, M.D.
S.A. Surgeon - Arthur Grove, M.D.
Medical Technologist - William B. Matthews, Jr.
Medical Technologist - Guy G. Cunningham
Biologist - Patricia A. Plunkert
Clerk-Stenographer - Patricia D. Williams
Clerk-Dictating Machine Trans. - Harriett C. Ottenstein
Clerk-Typist - Gertrude E. Wright
Laboratory Worker - Matthew P. Meadows

Section on Biophysical Applications:

Surgeon - Wayne Tobin, M.D.
Secretary (Stenographer) - Vernita Bergmeyer
Microbiologist - Dorothy J. Kitts

Section on Neuroradiology:

Research Biologist - Giovanni Di Chiro, M.D.
Secretary (Stenographer) - Carolyn E. Kline

Section on Clinical Applied Pharmacology:

Supervisory Research Pharmacologist - Richard L. Irwin, Ph.D.
Research Physiologist - Jay B. Wells, Ph.D.
Physiologist - Manfred M. Hein
Secretary (Dictating Machine Trans.) - Emma P. Dick

Surgical Neurology Branch:

Chief - Maitland Baldwin, M.D., M.S., F.R.C.S.
Associate Neurosurgeon - John M. Van Buren, M.D., M.Sc.
Medical Officer - Choh-luh Li, M.D., M.S.
Medical Officer - Ayub K. Ommaya, F.R.C.S.
Surgeon - Merwyn Bagan, M.D.
Surgeon - Fred G. Gieseke, M.D.
Surgeon - John M. Tew, M.D.
Surgeon - Eugene Flamm, M.D.
Surgeon - George Ojemann, M.D.

Surgical Neurology Branch (cont.):

Biologist - Levon O. Parker
Biologist - Rosemary C. Borke
Clerk-Dictating Machine Trans. - Helen M. Andregg
Clerk-Stenographer - Gertrude C. Grant
Clerk-Stenographer - Mary Haven
Clerk-Stenographer - Eleanor Lynn Crone

Section on Primate Neurology:

Surgeon - Thomas H. Milhorat, M.D.
Nurse Supervisor - Frances D.S. MacDonald, R.N.
Assistant Nurse Supervisor - Mildred L. Haddox, R.N.
Staff Nurse - Delta H. Trickett, R.N.
Staff Nurse - Blanche S. Suddreth, R.N.
Staff Nurse - Lorraine P. Huber, R.N.
Biological Laboratory Technician - Norman E. Mills
Clerk (Typing) - Celeste L. Sewell
Biological Laboratory Aid - Leo Jacobs
Medical Aid - Morris M. Williams
Biological Laboratory Technician - Calvin S. Hawkins
Medical Aid - Charles E. Sartor
Medical Aid - John Tolliver, Jr.
Medical Aid - Octavie W. Jacobs

Section on Neuroanesthesiology:

Visiting Associate - Tomio Ohta, M.D.

Section on Clinical Psychology:

Supervisory Research Psychologist - Herbert C. Lansdell, Ph.D.
Research Psychologist - Paul Fedio, Jr., Ph.D.
Psychologist - Beverly Vest
Psychologist - Nelly M. Urbach
Secretary (Typing) - Lillian L. Wease
Audiologist - Jean S. Baker

Section on Clinical Neuropathology:

Medical Officer - Igor Klatzo, M.D., M.Sc.
Staff Fellow - Lois E. Rasmussen, Ph.D.
Supervisory Medical Technician - Joseph T. Walker, Jr.
Biologist - Diane E. Smith
Biologist - Carlotta C. Clark
Biological Laboratory Technician - Ernestine G. Dye
Secretary (Typing) - Yvonne C. Miles
Medical Technician - Thelma R. Fletcher

Section on Child Neurology:

Medical Officer - Anatole Dekaban, M.D.
Surgeon - Owen Rennert, M.D.
Chemist - Diane E. Mizel
Medical Technologist - Rosemarie Thron
Secretary (Stenographer) - Linda G. Mahan
Medical Technician - Jan K. Steusing

Ophthalmology Branch:

Chief - Ludwig von Sallmann, M.D.
Visiting Associate - Rudolf Kern, M.D.
Surgeon - Vernon G. Wong, M.D.
Surgeon - William R. Green, M.D.
Surgeon - Ronald E. Carr, M.D.
Surgeon - George Spaeth, M.D.
Surgeon - Robert H. Collier, M.D.
Secretary (Stenographer) - Rita U. Durant
Clerk (Typing) - Harriett Murphy
Clerk-Stenographer - Dolores A. Cope
Biological Laboratory Aid - Barbara D. Bloom
Clerk-Stenographer - Delores A. Foltz

Section on Ophthalmology Physiology:

Physiologist - Michelangelo Fuortes, M.D.
Senior Surgeon - Peter Gouras, M.D.
Physicist - Ralph D. Gunkel, O.D.
Medical Aid - Krista Link
Electronic Development Tech. - James L. Jones
Clerk-Stenographer - Naomi Kay Boetker

Section on Ophthalmology Chemistry:

Research Chemist - Marc S. Lewis, Ph.D.
Chemist - Virginia Ann Aandahl

Section on Cell Biology:

Research Chemist - Sjoerd L. Bonting, Ph.D.
Research Chemist - Paul J. O'Brien, Ph.D.
Biologist - Mel Rose Canady
Biologist - Rubye J. Williams

Section on Cytology and Histopathology:

Research Biologist - Bolivar J. Lloyd, Jr.
Chemist - Patricia A. Grimes
Medical Technician - Eleanor M. Collins
Biological Laboratory Tech. - Julia Matthews
Biological Laboratory Tech. - Luther R. Dowell

Section on Ophthalmology Pharmacology:

Supervisory Pharmacologist - Frank J. Macri, Ph.D.
Biological Laboratory Tech. - Joseph G. Brown

Electroencephalography Branch:

Chief - Cosimo Ajmone Marsan, M.D.
Medical Officer - Kristof Abraham, M.D.
Surgeon - Bernard M. Abrams, M.D.
Supervisory EEG Technician - Maureen B. Berkeley
Supervisory EEG Technician - Emily B. Smith
EEG Technician - Helen T. Ellerbe
Secretary (Dictation Machine Trans.) - Elizabeth A. Lozada
Biologist - Samuel W. Cooper
EEG Technician - Ida Mae G. Hatter

Section on Clinical Neurophysiology:

Surgeon - Daniel A. Pollen, M.D.
Clerk-Stenographer - Janet Teti
EEG Technician - Raymond M. Glass

Laboratory of Neuroanatomical Sciences:

Chief - Alfred J. Coulombre, Ph.D.
Photographer - Edwin H. Moodhe
Secretary (Stenographer) - Ruth C. Jordan
Supervisory Biological Laboratory Tech. - Willie Perkins
Clerk (Typing) - Hilda M. Malcolm
Secretary (Typing) - Marie H. Dickerson
Biological Laboratory Tech. - Albert V. Cantu
Biological Laboratory Tech. - Melvin H. Carroll
Biological Laboratory Tech. - William N. Dorsey

Section on Experimental Neurology:

Senior Surgeon - Lloyd Guth, M.D.
Research Biologist - Jerald J. Bernstein, Ph.D.
Research Biologist - Stanley Jacobson, Ph.D.
Biological Laboratory Tech. - Janina D. Ziemnowicz
Biologist - William C. Brown

Section on Neurocytology:

Research Biologist - Keith C. Richardson, B.Sc., M.Sc.
Physiologist - Milton W. Brightman, Ph.D.
Surgeon - Thomas Reese, M.D.
S.A. Surgeon - Nicholas Lenn, M.D.
Biological Laboratory Tech. - Clara E. Mowry

Section on Functional Neuroanatomy:

Physiologist - Grant L. Rasmussen, Ph.D.
Medical Technologist - Edna P. McCrane
Biological Laboratory Tech. - Frank D. Nolan
Biologist - Helen J. Osborne

Section on Experimental Embryology:

Surgeon - G. Robert Delong, M.D.
Surgeon - Gordon W. Philpott, M.D.
Chemist - Jane L. Coulombre
Biologist - John L. Everly
Laboratory Worker - Kenneth M. Oglesbee

Laboratory of Biophysics:

Chief - Kenneth S. Cole, Ph.D., D.Sc.
Secretary (Stenographer) - Grace C. Ream
Clerk-Stenographer - Marilyn K. Kaplan

Section on Instrumentation:

Electronic Engineer - Leonard Binstock
Electronic Development Tech. - Herbert A. Walters

Section on Mathematical Biophysics:

Research Physicist - Richard Fitzhugh, Ph.D.
Research Physicist - Harold Lecar, Ph.D.

Section on Membrane Biophysics:

Supervisory Research Physiologist - Robert E. Taylor, Ph.D.
Research Physicist - Gerald M. Ehrenstein, Ph.D.

Section on Cellular Biophysics:

Research Physiologist - Daniel L. Gilbert, Ph.D.
Visiting Associate - Eduardo Rojas, Ph.D.
Physical Science Aid - Harold S. Davidson

Laboratory of Neurochemistry:

Chief - Donald B. Tower, M.D., Ph.D.
Secretary (Stenographer) - Vivian K. Arnold
Clerk-Dictating Machine Trans. - Sharon A. Ryan

Section on Amino Acid Chemistry:

Chemist - Edmund L. Peters

Section on Physiology and Metabolism:

Supervisory Research Chemist - Eberhard G. Trams, Ph.D.
Chemist - Carl J. Lauter

Section on Enzyme Chemistry:

Research Chemist - R. Wayne Albers, Ph.D.
Surgeon - Stanley Fahn, M.D.
Chemist - George J. Koval
Physical Science Aid - Eunice L. Summers

Section on Lipid Chemistry:

Medical Officer - Roscoe O. Brady, Jr., M.D.
Research Chemist - Andrew E. Gal, Ph.D.
Research Chemist - Julian N. Kanfer, Ph.D.
Chemist - Roy M. Bradley
Chemist - Oscar M. Young
Biological Laboratory Tech. - Alexander B. Wheaton
Clerk-Stenographer - Sylvia E. Simons

Laboratory of Neuropathology:

Medical Officer - Jan Cammermeyer, M.D.
Biologist - Iris Mercado
Biological Laboratory Tech. - Margaret G. Johnson
Editorial Assistant (Typing) - Jane T. Phelps
Biological Laboratory Aid - Sophia Grabinski

Laboratory of Neurophysiology:

Visiting Associate - Edward F. Evans, M.B., C.B., Ph.D.
Laboratory Worker Leader - Clifford A. Seay
Laboratory Worker - Harold E. Smith

Section on Spinal Cord:

Surgeon - Robert E. Burke, M.D.
Surgeon - Philip G. Nelson, M.D., Ph.D.
Surgeon - Raymond B. Wuerker, M.D.
Research Medical Officer - Thomas G. Smith, M.D.
Staff Fellow - Barbara O. Alving, Ph.D.
Physicist - Margaret W. Chapman
Biological Laboratory Tech. - William L. Beane
Secretary (Stenographer) - Ramona C. Duvall

Laboratory of Molecular Biology:

Chief - Ernst Freese, Ph.D.
Staff Fellow - Robert A. Lazzarini, Ph.D.
Staff Fellow - Richard Wax, Ph.D.
Research Chemist - Akira Yoshida, Ph.D.
Research Biologist - Elisabeth G.M. Freese, Ph.D.
Research Microbiologist - Austin L. Taylor, Ph.D.
S.A. Surgeon - Charles M. Cashel, M.D.
S.A. Surgeon - Michael Kaback, M.D.
Physical Science Tech. - Michiko Yoshida
Pharmacologist - Stuart L. Graham
Secretary (Stenographer) - Mary W. Taylor
Biological Laboratory Tech. - Cynthia McAlister
Clerk-Stenographer - Joyce H. Greene
Biologist - Carol J. Dunham
Physical Science Aid - Kerry R. Morrison

Laboratory of Perinatal Physiology - Puerto Rico

Medical Officer - Ronald E. Myers, M.D., Ph.D.
Research Psychologist - Gilbert Meier, Ph.D.
Veterinarian - Wendell Niemann, D.V.M.
Surgeon - John J. Schruefer, M.D.
Administrative Officer - Elizabeth W. Snowden
Administrative Assistant - Orlando Nieves
Librarian - Jorge Rivera-Ruiz
Chemist - Carmen L. Freixas
Photographer - Pedro Sola-Amadeo
Biologist - Carmen G. Ponce de Lugo
Biologist - Zaida I. Vega
Biological Laboratory Tech. - Maria L. Alvardao
Biological Laboratory Tech. - Luis C. Gonzales
Clerk (Typing) - Ruby C. Gittens
Clerk-Stenographer - Sara L. Mendez
Medical Aid - Calixto Jimenez-Lugo
Biologist - Luis R. del Rio

Laboratory of Perinatal Physiology - Puerto Rico (cont.)

Secretary (Dictating Machine Trans.) - Zara S. Powers
Supply Clerk - Carlos M. Rosario-Rivera
Biologist - Frank Resillez-Urioste
Clerk-Typist - Luis Rivera-Baez
Clerk-Typist - Ethel F. Butts
Clerk-Stenographer - Teresa A. Del Rio
Clerk-Stenographer - Carmen M. Ortiz
Biological Laboratory Tech. - Gladys Diaz de Torres
Biological Laboratory Tech. - Maria D. Lopez
Biological Laboratory Tech. - Carmelo Pagan-Blanchero
Clerk-Stenographer - Paulina T. Papp
Photographer - Luis Villafane-Santiago
Medical Technician - Juan B. Morales
Clerk-Typist - Luisa V. Sneed
Biological Laboratory Aid - Horacio Ramiriz-Pastrana
Guard - Manuel Solla-Hernandez
Messenger - Alfredo Perez
Facilities & Equipment Mechanic - Manuel Maldonado-Rosa
Animal Caretaker - Domisiano Melendez
Animal Caretaker - Israel Arce-Adames
Animal Caretaker - Gregorio Figueroa
Laborer - Cayetano Guzman
Animal Caretaker - Demetrio Melendez
Animal Caretaker - Inocencio Robledo
Animal Caretaker - Policarpio Quinones
Animal Caretaker - Juan R. Encarnacion-Colon
Animal Caretaker - Confessor Martis
Animal Caretaker - Manuel Rosado-Rosario
Animal Caretaker - Emilio Tolentino
Laborer - Aristides Quinones
Clerk-Stenographer - Teresa B. Morales

Laboratory of Perinatal Physiology - Puerto Rico (Islands)

Supervisory Research Biologist - Carl Koford, Ph.D.
Research Biologist - John G. Vanderbergh, Ph.D.
Construction and Maintenance Superintendent - Carlos R. Nagel
Clerk-Stenographer - Susan J. O'Higgins
Carpenter- Jacinto S. Rosado
Animal Caretaker - Victor A. Bracero-Pagan
Carpenter Helper - Jose A. Flores
Animal Caretaker - Angel Figueroa-Velez
Animal Caretaker - Sergio Rivera-Vasquez
Animal Caretaker - Israel Cordero
Animal Caretaker - Estaban Rivera
Laborer - Abraham Rosado
Animal Caretaker - Rafael Rivera-Correa

ANNUAL REPORT OF THE SCIENTIFIC DIRECTOR

OF THE

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

July 1, 1964 through June 30, 1965

The Intramural Research activity of NINDB has continued to operate on a relatively stable base during this twelve month period. Comparisons with the previous Annual Report should take into account the eighteen month base of that report due to the shift from calendar to fiscal year. The ten Laboratories and Branches of the Intramural Program in Bethesda continue to carry 242 positions. One hundred and seven (107) of these are professionals at the level of GS-11 or higher, or equivalent. Using this level to divide professional from non-professional employees we have an average of 1¼ non-professionals per GS-11 or higher employee. Space available to the Program has increased by about 900 square feet or approximately 3 per cent. The Intramural Budget taken on a twelve month base has increased by 2.8 per cent.

In some areas of the Program there are many well-qualified young recruits available, in others recruiting appears to be more difficult. The competitive position of NIH with regard to recruiting varies in the different specialties. One of the most important measures of success of the Program is its ability to attract good people and this appears to be as good as ever.

The present Acting Associate Director feels that it is important to the Program that a permanent Scientific Director be found and has asked to be relieved of his present duties by January 1, 1966. A number of candidates have been suggested for this position and two have been interviewed.

Twenty new people have been employed during the year to replace those who have left. Thirteen of these are clinical or research associates or staff fellows. The remaining seven have been given Civil Service status. Thus, there are now seven fewer temporary positions available to the Program; without further expansion there will be a smaller turnover of personnel next year. One result of these forces is the increasing average age of the Intramural Staff. Of the 107 GS-11 or higher employees the average age last year was 36.3 years, while the average for the current reporting period is 37.2. This trend will presumably continue until new space and positions are made available or until a plateau is reached of very mature individuals.

In order to combat this trend an effort should be made to replace permanent employees who leave with temporarily filled positions wherever possible.

Some additional space may become available when the new Clinical Center Library quarters are occupied, probably early in 1967. Completion of the new NIMH/NINDB Building 36, scheduled for early 1968, will provide a large increase in space for the Intramural Research Program. The very great potential represented by this space increase must not be wasted by assigning it too quickly to existing Laboratories and Branches. Slow and steady expansion will have the maximum vitalizing effect on this research organization. There is an eternal conflict between the need to make the best use of available space and the need to keep it from slipping away.

The number of research projects reported this year is 192 in comparison to the 170 reported last year despite the 50% longer reporting period of the previous year. While the greatest assets of NIH continue to be the opportunity for collaboration, bringing a number of different specialized disciplines to bear on a single research objective, evidence that the Intramural Program is availing itself of such opportunities is indicated by the fact that 101 of the research projects currently active involve collaboration with other Laboratories and Branches within NINDB, within NIH, and with outside organizations. About half of these represent collaboration with other Institutes, one-third show collaboration with Laboratories outside NIH.

The number of projects reported as completed and/or terminated is 59. This number is difficult to interpret but represents a shift of space and manpower to new projects in the continuing competition between new and old research operations.

The newest addition to the NINDB Intramural Research Program is the Laboratory of Perinatal Physiology in Puerto Rico under Dr. Ronald E. Myers. This Laboratory, formerly under Dr. William Windle, has not been included in the above figures, which showed no change in space or positions, because it is being transferred from the Collaborative and Field Program. The Laboratory occupies temporary quarters in the U.S. Public Health Service Quarantine Station in San Juan and operates several islands around Puerto Rico. The Laboratory is scheduled to be housed in a new building to be erected in the Centro Medico in close proximity to the new University of Puerto Rico Medical School and other functionally related organizations.

It is difficult to recognize the most important research achievements of the Intramural Research group, perhaps the best judges of this are the Laboratory and Branch Chiefs themselves. The annual accomplishments and plans for future research are described in summaries preceding the individual reports of each Laboratory and Branch.

INTRAMURAL RESEARCH
NINDB

Program	Persons		$ In Thousands		Space Sq. Ft.		Beds	
	FY-64	FY-65	FY-64	FY-65	FY-64	FY-65	FY-64	FY-65
Office of Assoc. Director	26	26	250	243	2494	2920	---	---
Miscellaneous	--	--	264	103	--	--	---	---
Sub total	26	26	514	346	2494	2920	---	---
Med. Neuro.	25	25	290	360	2675[1]	2641[1]	26	26
Surg. Neuro.	51	51	600	605	5425[1]	5488[1]	26	26
EEG	12	12	107	130	1697[1]	1697[1]	---	---
Ophthalmology	34	34	394	396	3800[1]	3800[1]	26	26
Sub total[2]	122	122	1391	1491	13597	13626	78	78
Biophysics	13	13	175	212	1530	2178	---	---
Neuroanatomy	30	30	293	348	7130	6907	---	---
Mol. Biol.	15	15	162	194	3328	3328	---	---
Neurophysiol.	11	11	149	153	2035	2035	---	---
Neuropathology	5	5	54	57	1239	1211	---	---
Neurochemistry	20	20	258	264	3200	3205	---	---
Sub total[3]	94	94	1091	1228	18462	18864	78	78
TOTAL	242	242	2996	3065	34553	35410	---	---
Perinatal Physiology	67	67	737	746	20461 + 109acre	18289 + 109acre	--	---
GRAND TOTAL	309	309	3733	3811	55014 + 109 acre	53699 + 109acre	78	78

On April 16, 1965, the Laboratory of Perinatal Physiology was transferred from the Collaborative and Field Research Area to the Intramural Research Area.

[1] These figures do not include Clinical Center space (patient wards and surgical wing, Building 10A) occupied by the Branches indicated.

[2] Clinical Program

[3] Basic Program

ANNUAL REPORT
July 1, 1964 through June 30, 1965
MEDICAL NEUROLOGY BRANCH, IR
National Institute of Neurological Diseases and Blindness

W. King Engel, M.D.
Chief, Medical Neurology Branch

Clinical Investigation Program

Introduction: The function of this program is to apply the most promising basic research techniques to the clinical problems of the patients. The essentiality of an inter-related multi-dimensional attack on the chosen target diseases is to be emphasized. Added to the techniques of histochemistry and tissue culture have been biochemistry and immunology, but in quite modest forms due to limitations of space and personnel. The techniques of electron-microscopy and autoradiography have been begun only on a collaborative basis, due to acute lack of space for these important investigations. We are very appreciative of the collaboration received in these and other techniques. It is obvious that to have a balanced clinical investigative program, each of these techniques must be provided for more adequately.

For the clinical investigations, 275 patients were admitted for a total of 6,786 patients days, and 744 out-patients were seen. There were 296 muscle biopsies obtained.

The clinical neurologists carried a considerable service responsibility. They provided 356 consultations to other departments, and performed the indicated myelograms, pneumoencephalograms, and cerebral angiograms on those patients.

The two-year approved residency training program in clinical neurology has continued; medical students and residents from Howard University were taught clinical neurology weekly; and technicians and investigators came as guest workers to learn clinical research techniques in neurology and especially the application of enzyme histochemistry to human neuromuscular disease.

Myopathies: A completely new classification of the myopathies based primarily on metabolic aspects has been developed. Treatment of myopathies associated with collagen-vascular disease has been rather successful in some patients, and in certain cases resulted from reduction of the medication they were on; appropriate treatment was chosen on the basis of clinical judgment. A symposium on Current Concepts of the Myopathies

has been guest-edited and seven chapters written by our neurologists; it is also being published as a separate monograph. Chapters summarizing our total experience on "Histochemistry of Neuromuscular Disease" have been written for two texts. An even more comprehensive Atlas summarizing muscle biopsy histochemistry of more than 1,000 patients with neuromuscular disease is being prepared. Many new histochemical findings have been described, compared, and contrasted in a variety of myopathies including hereditary dystrophies, collagen-vascular myopathies, endocrine myopathies, and certain rare myopathies. Studies have been made of selective fiber involvement according to histochemical type in certain myopathies. Basic histochemical mechanisms have been analysed in human (and some animal) skeletal muscle, including direct Nitro-Blue Tetrazolium binding, the phenazine methosulfate shunt, and various ATPase reactions. A method for isozyme histochemistry of lactate dehydrogenase has been devised. Detection of subtle abnormalities with the histochemical reactions has pointed up the great need for more studies of normal human muscle, which we are commencing. Molecular abnormalities of myoglobin and muscle lactate dehydrogenase were found not to be specific for muscular dystrophy, nor was decrease in total body K^{40}. Turnover of several serum proteins was studied; excessive catabolism of γ-globulin was found in nearly all patients with myotonic dystrophy and in none of the other neuromuscular disorders studied. Accuracy of detecting assymptomatic carriers of muscular dystrophy was evaluated using serum creatine phosphokinase and muscle biopsy histochemistry studies. Tissue culture was used to study various aspects of skeletal muscle cell biology, both of chick embryo and, to a lesser extent, of human tissue. Electron-microscopy of abnormal human muscle is in the early stages. A technique has been devised for histochemical control of the specimens at several stages of their processing for electron-microscopy.

Episodic Weakness: A new classification of the periodic paralyses and non-dystrophic myotonias has been developed, based on the provocative and therapeutic effects of various ionic unbalancing tests. The tests are designed to determine the identity or non-identity of these various clinical conditions, based on the more accurate metabolic parameters. The therapeutic trials indicated by the test results are being run in these conditions. Forearm studies are planned for the future to study more selectively muscle metabolism in these patients. Muscle biopsies obtained between and during attacks have been investigated histochemically, and electron-microscopy is in progress. Histochemical studies make it doubtful that structural changes in the muscle fibers are responsible for the weakness in the initial part of the attack. The new pathological entity "mitochondrial aggregate" has been described in patients with these conditions, and its specificity is being analysed. In a patient with succinylcholine-induced paralysis, the low serum cholinesterase was found by electrophoresis to be associated with a newly recognized selective absence of the fastest two cholinesterase bands.

Myasthenia Gravis: A number of immunologic techniques have been set up to study immunologic aspects of myasthenia gravis, as well as of myopathies in collagen-vascular disease. Immunologic techniques are also being used to study protein abnormalities in other neurologic disorders, such as ataxia telangiectasia and the progressive peripheral neuropathy of primary amyloidosis. More emphasis on studies of the altered cellular aspects of immunity in neurologic disease is planned. In myasthenia gravis, with a very careful fluorescent antibody study, the muscle-binding factor in myasthenia gravis was not found to be bound to the neuromuscular junctions. Clinical and immunologic aspects of non-myasthenic patients with thymona are being studied to seek subclinical evidence of myasthenia. Histochemistry of muscle biopsies from 38 myasthenia gravis patients showed that virtually all were abnormal, with denervation atrophy and type II fiber atrophy (a newly described histochemical change) being far more common than muscle fiber necrosis of cellular reactions (lymphorrhages).

Amyotrophic Lateral Sclerosis (ALS), and Other Diseases Affecting the Lower Motor Neuron: A monograph is in preparation. The first part is a review of the entire literature on ALS. The second part covers our special investigations, clinical and basic, of ALS. Clinical data on 200 patients are being analysed by an inverted index system using a specially developed vocabulary. In the first 100 patients, 10% were found to have neoplasms. In about 150 patients, approximately 25% have abnormalities of carbohydrate metabolism, the mechanisms of which are being sought. Intolerance to amino acids and other metabolites is being sought, as are abnormalities in protein, lipid, and cyanocobalamine metabolism. For therapeutic trials, an apparatus giving reproducible quantitation of muscle strength has been developed and double-blind evaluation of several methods of treatment (as well as placebos) is nearly completed. Metabolic parameters of biopsied motor neurons from ALS patients are being studied by autoradiography, histochemistry, and biochemistry; and neuronal ultrastructure is being explored by electron-microscopy. Control neuronal material is obtained from patients with other diseases and from normal apes. The histochemistry of animal motor neurons in tissue culture is being studied. The changes in histochemistry and lactate dehydrogenase isozymes in animal muscle after denervation have been described.

Neuroradiology Section

Radiographic Diagnosis: A project aiming at an appraisal of the vascularization of spinal cord lesions is under way. Angiography with selective catheterization and with subtraction is being carried out for this purpose in patients with arteriovenous

malformations and tumors of the spinal cord. The Atlas of Pathologic Pneumoencephalographic Anatomy is nearing completion. A cooperative project is being initiated on growth hormone effects in dwarfs. Repeated sella-turcica measurements will be taken in the patients so treated. A reevaluation by computer techniques of the angiographic patterns of superficial cerebral veins in the two hemispheres is under way. The aim of this project is to re-evaluate on a very large clinical material the previous finding that the angiographic patterns of superficial discharging veins in the two hemispheres are different, and that a relationship of these morphological differences with the cerebral functional dominance exists. Further experience is being gained with the useful refinement of pneumoencephalography developed in this section, namely "axial transverse encephalography".

Radiation Dosimetry: A system is being evaluated to measure secondary radiation from irradiated residual x-ray opaque material in the spinal canal. Among the solid-state radiation detection devices, the thermoluminescent lithium floride (LiF) crystals have been chosen.

Isotopic Diagnosis: A clinical comparison of radio-iodinated serum albumin and technetium ^{99m} pertechnetate as brain scanning agents is under way. The aim of this project is to evaluate the relative merits of two widely used radioactive tracers, RISA and technetium ^{99m} pertechnetate. This appraisal will be as objective as possible in clinical material, due to the fact that the double isotope-double scan technique is being used, i.e., two tracers are injected in the same patient, each injection being followed by a scan. Radioactive isotopes, especially technetium ^{99m} pertechnetate, and brain scanning have been used to evaluate the patency and morphology of cerebrospinal fluid shunts. For the first time, the shunting paths have been actually visualized. Further experience has been accumulated in isotope-ventriculography and isotope-cisternography. These have proven to be diagnostic tools permitting the morphologic and dynamic study of the cerebrospinal fluid pathways more accurately than was ever possible before with any other diagnostic test. With the adjunction of our new multiprobe detecting device - the "Tetrascanner" - the mapping and timing of the cerebrospinal fluid circulation is being studied in great detail. Stress is placed upon the pathologic dynamics of the same fluid. Virus-induced experimental brain tumors, namely intracranial sarcomas and gliomas, have been produced in dogs. This appears to be an ideal biological system for a comparative evaluation of the many radioactive tracers used or potentially usable in human brain scanning. Such potential tracers will be screened in these animals. The observation that technetium ^{99m} pertechnetate has a very high specificity for salivary glands has brought about a project in which salivary gland scanning is being evaluated. Emphasis will be put upon salivary gland tumors and other

salivary pathology. The general plan has been prepared and a few chapters have been written of a monograph dealing with the newest aspects of brain scanning.

Neuropharmacology Section

This section has continued its research program in relation to the physiology and pharmacology of muscle. We have found that at least certain smooth muscles, as well as slow striated muscles, develop contracture in response to calcium deprivation. The response may not be related directly to electrical activity. The findings thus indicate that intracellular calcium movements may control tension development. These studies have necessitated the recording of electrical activity in slow skeletal and smooth muscle; new methods for these measurements have been developed and used. The ionic requirements for relaxation of slow skeletal muscle have also been studied, and much data have been obtained. The meaning of these data is not known as yet and they thus remain descriptive.

A comparative pharmacological study of fast and slow types of twitch muscle has continued to a limited extent. The study involves further instrumentation development. A study of the genetics of serum cholinesterase has been made in a family with serum cholinesterase abnormality.

Mechanical properties of muscle have been studied as a prerequisite to the evaluation of drug action. Unexpected differences have been found between the elastic components of fast and slow twitch type muscles. The mechanical properties derived from dynamic elastic responses were analysed in terms of physical elastic models. A suitable technique for limb joint fixation in animals has been developed. The method provides a modified mechanical environment for the muscle in the presence of an intact neural apparatus. Immobilization in slower muscles produced atrophic and mechanical changes. These studies are in progress and are to be continued to include the faster responding muscle.

Perfusion of isolated portions of the cerebral ventricles in cats with pharmacologic agents has been further refined. The effects of various agents on cerebral physiology have been recorded, and the pathophysiology of the induced myoclonic jerks investigated. Future plans call for more precise localization of the pharmacologic agents by use of radioisotopic and fluorescent markers.

Serial No. NDB(I)-65 MN/OC 1193 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Radioautography Applied to the Study of Neurologic Disease

Principal Investigator: Leon A.H. Hogenhuis, M.D.

Other Investigators: Beatrix Kopriwa, Ph.D.

Cooperating Units: McGill University

Man Years:
Total 0.2
Professional: 0.2
Other:

Project Description:

Objectives: To apply radioautographic techniques with the use of purine, pyrimidine, amino acid and lipid precursors to the study of diseases affecting the nervous system.

Methods Employed: Radioautography was employed utilizing the excellent resolution afforded by the low energy ß-radiation of triated compounds; the Kodak NTB 2 emulsion was used for processing the tissue with the dipping technique of Leblond and Kopriwa.

Patient Material: Brain biopsy specimens from patients suffering from one of the clinical varieties of motor neuron disease (amyotrophic lateral sclerosis, bulbar paralysis, progressive muscular atrophy or infantile spinal muscular atrophy) were obtained from patients admitted to the Medical Neurology Branch with such a motor neuron disorder. The central nervous system was exposed to a tritiated RNA precursor prior to the brain biopsy procedure by intracysternal injections of the compound.

Major Findings: An extensive study of the uptake pattern of the intracysternally administered labeled precursor in different

parts of the CNS of a series of rabbits, revealed a spatiotemporal optimum of one hour for uptake in the nucleus of large neurons and an optimum of two days for uptake in the cytoplasm of the large neurons. This technique was applied to humans after adjustments were calculated according to the observed kinetic pattern in the experimental animal. Preliminary results have confirmed the validity of these kinetics for the human. The technique was applied to one case of infantile spinal muscular atrophy, one adult case of familial amyotrophic lateral sclerosis with bulbar and supra bulbar signs and one adult case of progressive muscular atrophy with bulbar and suprabulbar signs. Specimens are in the process of being analyzed for abnormalities in the uptake of the precursor in the purine or pyrimidine moieties of the neurons involved in the disease process.

Significance to Neurologic Research: Abnormalities at the cellular and subcellular level can readily be studied with this technique and the site of the lesion in the affected structural elements of the central nervous system can be identified more specifically, especially in regard to RNA metabolism.

Proposed Course of Project: Patients with this type of selective involvement of the large neurons will continue to be admitted for this study to participate in the research program outlined above. Clarification of the site of the lesion at cellular and subcellular level will induce further efforts to find a rationale for treatment of this still incurable, invariably fatal disorder.

Part B included No

Serial No. NDB(I)-65 MN/OC 1192 (c)
1. Medical Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Electron Microscopic Studies of Skeletal Muscle and Neurons

Principal Investigator: Jerome S. Resnick, M.D.

Other Investigators: W. King Engel, M.D.
Keith C. Richardson, Prof.

Cooperating Units: NINDB/LNS

Man Years:

Total:	0.7
Professional:	0.6
Other:	0.1

Project Description:

Objectives: To study early fine cellular changes in human muscle in diseases that are confined to muscle alone, as well as in conditions which produce secondary alterations in muscle such as denervating and metabolic diseases. To study subcellular changes in certain diseases affecting neurons.

Methods Employed: A portion of biopsied muscle is tied to a glass rod to maintain resting length and immediately fixed in cold buffered osmium tetroxide for araldite embedding. Adjacent biopsy material is immediately frozen for cryostat sections and incubated in various histochemical media as described in another project. Some stained cryostat sections are ready within an hour after the biopsy. These sections can be used as a guide to determine which of the biopsies contain light microscopic features of special interest and, in many instances, thereby serve as a guide for choosing material appropriate for electron microscopic study. Biopsy specimens of human brain tissue are fixed and embedded for electron-microscopy.

Patient Material: Patients with various myopathies and neurogenic muscular weakness are the major source of material. Congenital non-progressive neuromuscular dysfunction without

evident pathologic changes on light microscopy and histochemistry (so-called benign congenital hypotonia) are also under study. When available, tissue from asymptomatic carriers of progressive genetic myopathies is taken for study. Brain biopsies are obtained from patients with amyotrophic lateral sclerosis and ones with certain forms of progressive dementia.

Major Findings: In the few months since this project was begun attention has been focused on material from patients with denervating disease in which concentric alteration of the muscle cells is present, the "target fibers" previously described. These target fibers may represent the earliest response of human muscle cells to impaired trophic influence from motor neurones. Preliminary ultrastructural examination shows the earliest recognizable change in target fibers to be in the myofibrils near the center of the fiber, with myofibrillar narrowing and loss of the normally distinct alternating A and I band pattern. As histochemical studies have suggested, at this stage, there is a relative increase per unit volume of mitochondria, with clumping. The mitochondria individually appear normal. The nature of the alteration and risidual material in the most severely affected (central) areas is being further elaborated. Mitochondria are not seen in the center of the muscle fiber in the fully evolved target. Similarities are noted with the previously described hereditary "central core disease" and this raises the possibility that central core disease might be a manifestation of incomplete innervation during fetal life, rather than a myopathy in the usual sense of the word.

Significance to Neurologic Research: In the case of target fibers, more detailed structural and chronologic observations on this response of the muscle cell should broaden our knowledge as to the role that the nervous system plays in maintaining muscle viability, and may lead to the incrimination of a central nervous system role in certain diseases that at this time are generally considered to be confined to muscle cells only. Studies of patients with benign congenital hypotonia and asymptomatic carriers of muscular dystrophy, if positive, can provide clues as to which portions of the complex metabolic system of muscle are primarily involved and thereby serve as a guide for future biochemical studies.

Proposed Course of Project: Further studies that combine immunologic techniques and autoradiography with electron microscopy are being contemplated, so as to supplement the morphologic approach with pertinent studies of cellular dynamics.

Part B included No

Serial No. NDB(I)-65 MN/OC 1191 (c)
1. Medical Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Immunological Abnormalities of Neurologic Disease.

Principal Investigator: Dale E. McFarlin, M.D.

Other Investigators: Mahlon Barlow, M.D.
Thomas Waldman, M.D.
Jerome Block, M.D.
George Glenner, M.D.

Cooperating Units: NCI/ODIR; NCI/MB; NIAID/LEP

Man Years:

Total	1.3
Professional:	0.8
Other:	0.5

Project Description:

Objectives: To apply immunological techniques to the study of diseases affecting the nervous system.

Methods Employed: Standard immunochemical techniques were employed. In addition, preliminary use of tissue culture technique was begun.

Patient Material: Specimens of peripheral blood, bone marrow, muscle, peripheral nerve, thymus and other tissue were obtained from patients with neurological disease admitted to the Medical Neurology Branch.

Major Findings: A laboratory for immunological research was established. Banks of serum and various tissues were started. Studies of familial amyloidosis, ataxia teleangectasia and collagen-vascular diseases were initiated. In familial amyloidosis no evidence of a serum protein abnormality or of plasma cell dysfunction was found. Deficiency of IGA globulin in ataxia teleangectasia, reported by others, was confirmed and studies of the mechanism of this defect were begun.

Significance of Neurologic Research: Absence of a disorder

of plasma cell function in familial amyloidosis suggests that amyloid is produced by a different cell line. Clarification of this point would provide a basis of directing therapy aimed at inhibiting amyloid formation. Likewise, elucidation of the defect of protein metabolism in ataxia teleangectasia may disclose the chemical abnormality responsible for impaired nervous system function in this disease.

Proposed Course of Project: The present studies will be expanded. Specific metabolic and tissue culture studies are planned in the above conditions.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Somers, J.E.: Myelin antibodies in experimental allergic encephalomyelitis. Neurology 14: 232-235, Mar. 1964.

Somers, J.E., Kanfer, J.N., and Brady, R.O.: Immunochemical studies with gangliosides. II. Investigations of Structure of Gangliosides by the Hapten Inhibition Technique. Biochem. 3: 251-253, Feb. 1964.

Honors and Awards relating to this project: None.

Serial No. NDB(I)-63 MN/OC 1036(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Amyloidosis

Principal Investigators: Irwin A. Brody, M.D.
Dale E. McFarlin, M.D.

Other Investigators: Paul T. Wertlake, M.D.
W. King Engel, M.D.

This project is incorporated in No. 1191

Serial No. NDB(I)-65 MN/OC 1190 (c)
1. Medical Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Myasthenia Gravis

Principal Investigators: Dale E. McFarlin, M.D.
W. King Engel, M.D.

Other Investigators: Mahlon H. Barlow, M.D.
Arthur J.L. Strauss, M.D.
Wayne E. Tobin, M.D.
John Vasko, M.D.
Joseph H. McLaughlin, M.D.

Cooperating Units: NHI

Man Years:

Total	0.75
Professional:	0.50
Other:	0.25

Project Description:

Objectives: To apply histochemical pharmacological, electrophysiological and immunological techniques to the investigation of the pathogenesis of myasthenia gravis. Emphasis was placed on immunological study because: 1. Immunological abnormalities have recently been demonstrated in the serum of myasthenic patients. 2. Thymic pathology in patients with myasthenia is well known; it is now felt that this gland has major immunological functions.

Methods Employed: Sera from patients with myasthenia gravis were studied by a variety of immunological techniques including agar diffusion, immunoelectrophoresis, latex agglutination, and immunofluorescence. Specimens of muscle, and in some cases thymus and thyroid, were studied using immunofluorescent, histochemical and routine histological techniques.

Patient Material: Sera, muscle, thymus and other tissue were obtained from patients whose clinical status had been characterized by the NINDB staff.

Major Findings: (1) The properties of myasthenic muscle have been characterized histochemically. (2) Four types of abnormalities were seen: a. "Fiber necrosis", b. "Denervation atrophy", c. "Lymphorrhages" and d. "Type II atrophy". At least one change was found in 97% of all myasthenic patients. (3) No reactivity between serum from myasthenic patients and the neuromuscular junction has been demonstrated by immunofluorescent techniques.

Significance to Neurological Research: (1) Techniques previously employed have revealed muscle pathology in less than fifty per cent of specimens from myasthenic patients; however, in the present study both a higher incidence and a wider variety of abnormalities were demonstrated. Characterization of the muscle pathology in myasthenia gravis may provide a clue as to the pathogenesis (es) of the neuromuscular junction defect. (2) Demonstration of nonreactivity between immunoglobulins from myasthenic patients and the neuromuscular junction is in contrast to the report of binding at "end plate-like" structures by one group of investigators who imply that a reaction between abnormal serum proteins and receptor substance is responsible for the pathophysiology of myasthenia gravis. The data obtained in this study suggest that relationship between immunological abnormalities and the physiological defect is by some means other than a direct binding at the end-plate.

Proposed Course of Project: The number of patients studied will be expanded and attention focused on post-thymectomy patients. A few cases of neonatal myasthenia will be studied, if available. Techniques involving the study of cellular immunity are to be added, which should facilitate characterization of the thymic abnormality in myasthenia gravis.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Brody, I.A. and Engel, W.K.: Denervation of muscle in myasthenia gravis: Report of a patient with myasthenia gravis for 47 years and histochemical signs of denervation. Arch. Neurol. 11: 350-354, Oct. 1964.

Engel, W.K. and McFarlin, D.E.: Skeletal pathology in myasthenia gravis -- Histochemical findings. Trans. NY Acad. of Sci. (In Press).

McFarlin, D.E., Engel, W.K., and Strauss, A.J.L.: Does myasthenic serum bind to the neuromuscular junction? Trans. NY Acad. of Sci. (In Press).

Honors and Awards relating to this project: None.

Serial No. NDB(I)-62 MN/OC 919(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Histology of Biopsied Skeletal Muscle in Myasthenia Gravis

Principal Investigators: W. King Engel, M.D.
Irwin Brody, M.D.

Other Investigators: None

This project incorporated in No. 1190

Serial No. NDB(I)-65 MN/OC 1189 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Episodic Weakness

Principal Investigators: Jerome S. Resnick, M.D.
W. King Engel, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.4
Professional: 0.4
Other:

Project Description:

Objectives: To define more clearly those disorders affecting the neuromuscular apparatus which present primarily with episodic weakness or paralysis. Attention is to be directed toward those conditions in which evidence suggests that the main site of intermittant dysfunction is somewhere within the following portions of the neuromuscular system: motor end plate region; sarcolemmal membrane; and sarcolemmal - sarcoplasmic reticulum - myofibrillar complex (excitation contraction coupling mechanism). Further experimentation is to be done with agents which are either provocative or therapeutic with respect to periodic paralysis syndromes with a view to obtaining more information regarding pertinent metabolic pathways.

Methods Employed: The techniques of clinical investigation (including electromyography and clinical biochemistry), muscle biopsy with samples for histochemical analysis, electron microscopy, and biochemical study are delineated in other projects. Provocative loading tests and therapeutic trials are necessary.

Patient Material: Patients of all ages are admitted to the Medical Neurology Branch for this project if they have intermittant muscular weakness associated with familial periodic

paralysis, hypo- or hyperkalemic; isolated examples of periodic paralysis with potassium disturbance; thyrotoxic periodic paralysis; paramyotonia congenita; or myotonia congenita. (Patients with myasthenia gravis are part of another project.)

Major Findings: Initial success has been obtained in preventing hypokalemic attacks by parenteral thiamine, confirming a report by Japanese workers. Other patients will be studied in a double-blind fashion to further confirm this treatment. The mechanism of thiamine's benefit will be studied by exploration of carbohydrate and pyruvate metabolism.

Na and K fluxes across the red cell membrane are being conducted with Dr. John Parker, NHI, to seek abnormalities of these systems in periodic paralysis.

A new classification of periodic paralyses and myotonia congenita has been postulated on the basis of ionic unbalancing tests and is being tested for validity. It is designed to give insight into the pathogenesis of the attacks and the means of treating them. These tests will also help settle the controversy regarding the identity or non-identity of certain entities such as hyperkalemic periodic paralysis (adynamia episodic hereditaria) and paramyotonia congenita.

Muscle biopsies between and during attacks are being studied by histochemical, biochemical, and electron-microscopic techniques, as described under those projects.

Significance to Neurologic Research: The existence of rare individuals with an endogenous metabolic defect which results in intermittant interruption of the normal neuromuscular excitatory pathway provides a unique model, study of which should provide clearer insight into the roles that carbohydrate metabolism and electrolytes play in muscular contraction. More effective therapeutic and prophylactic agents might thereby result for patients so afflicted

Proposed Course of Project: Complete studies mentioned under "major findings", with more emphasis on electron-microscopic analysis of the muscle, and radioisotopic studies of ions and metabolites affecting the attacks of weakness.

Begin forearm perfusion studies to challenge and analyse the muscle more directly.

Part B included. Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Resnick, J.S.: Episodic Muscle Weakness, Chapter 5. A Symposium "Current Concepts of the Myopathies" in Clinical Orthopaedics and Related Research, pp. 63-73, 1965.

Honors and Awards relating to this project: None.

Serial No. NDB(I) MN/OC 1046 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Diencephalic Seizures - Relation to Periodic Disease

Principal Investigator: Richard N. Rovner, M.D.

Other Investigators: None

This project is completed.

Part B included Yes

Serial No. NDB(I) MN/OC 1046 (c)

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Rovner, R.N.: Diencephalic seizures in relation to periodic disease. Trans. Am. Neurol. Ass. (In Press).

Honors and Awards relating to this project: None.

Serial No. NDB(I)-63 MN/OC 1045 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Nerve Conduction Velocity in Treated and Untreated Adrenal Insufficiency Patients

Principal Investigators: John R. Warmolts, M.D.
Robert I. Henkin, M.D.

Other Investigators: Albert A. Carr, M.D.
John R. Gill, M.D.
Frederick C. Bartter, M.D.

This project is completed

Serial No. NDB(I)-63 MN/OC 1043 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: EEG Abnormalities in Acute Leukemias

Principal Investigators: Richard N. Rovner, M.D.
Kristof Abraham, M.D.

Other Investigators: None

This project is completed.

Serial No. NDB(I)-63 MN/OC 1044(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Study of Afferent Innervation of Muscle in Parkinsonism

Principal Investigator: John R. Warmolts, M.D.

Other Investigator: John M. Van Buren, M.D.

The project is completed

Serial No. NDB(I)-63 MN/OC 1042(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Subtraction Technique: Experiences with Application to Transfemoral Renal Arteriography

Principal Investigators: S. David Rockoff, M.D.
Walter A. Camp, M.D.

This project is completed

Serial No. NDB(I)-63 MN/OC 1039 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Amyotrophic Lateral Sclerosis (ALS) and Other Lower Motor Neuron Diseases

Principal Investigators: W. King Engel, M.D.
Leon A.H. Hogenhuis, M.D.

Other Investigators: E. Arnold Carmichael, M.D.
William J. Collis, M.D.
Gerald N. Gold, M.D.

Cooperating Units: None

Man Years:
Total: 5.5
Professional: 4.8
Other: 0.7

Project Description:

Objectives: To find a means of arresting ALS.

Methods Employed: Review of the world literature on this disease and on studies related to motor neuron function and malfunction.

Analyse findings in our own series of about 200 ALS patients by careful clinical and laboratory examinations. Store and analyse in an inverted index data processing system.

Double blind therapeutic trials, the efficacy of which is judged by clinical testing, functional evaluation, and serial quantitative evaluation of muscle function using an apparatus designed by us.

I.V. pyruvate and I.V. arginine tolerance tests.

Carbohydrate metabolism studies - glucose, tolbutamide, and insulin tolerance tests.

Analysis of muscle biopsies by histochemical, electron-

microscopic, and tissue culture techniques, as described in other project reports.

Analysis of brain biopsies by histochemistry, electron-microscopy, and autoradiography, as described in other project reports, as well as by cholinesterase assay and terminal bouton staining.

Monkey spinal cord and motor cortex obtained by biopsy are being studied for comparison with human disease material.

Biochemical assays of serum fatty acids, of turnover of serum protein fractions, and of factors related to vitamin B_{12} metabolism.

Growth of animal motor neurons in tissue culture to study their characteristics in vitro.

Patient Material: Medical Neurology Branch patients.

Major Findings: Because it is somewhat new to apply a variety of well controlled basic science methods to ALS, the techniques developed for use in patient material are, in a sense, results. Definitive results of the various techniques will not be available for a year or two. A few preliminary items can be recorded.

Among the first 130 ALS patients, 10% were found to have an associated neoplasm, but additional patients are not showing this high incidence.

About 25% of the ALS patients have diabetic glucose tolerance tests. For comparison, our patients with other diseases were studied, with a similar incidence of diabetes being found in muscular dystrophy, late-onset myopathy, peroneal muscular atrophy (Charcot-Marie Tooth disease), and myotonic dystrophy.

Results of the I.V. pyruvate and I.V. arginine tolerance tests are not yet available.

Repeated quantitative evaluation of muscle function, as well as of the history and neurologic exam, is giving a more detailed account of the natural course of ALS.

Three agents have been tried in a double blind therapeutic trial, but the code has not yet been broken to evaluate the results. These tests will give not only the results with these

3 substances, but also guide our planning of additional therapeutic trials in ALS.

Turnover of several serum proteins is normal, as are blood fatty acid levels.

Analyses of biopsies of muscle and brain are discussed in projects related to the special techniques used.

Significance to Neurologic Research: Should any of the studies in progress or planned lead to a method of arresting ALS, they would not be without significance. The special types of analyses of biopsied muscle and brain will give new details on that pathological material.

Proposed course of project: To more fully develop some of these studies that have barely been begun (see methods).

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Norris, F.H., Jr., and Engel, W.K.: Neoplasia in Patients with Amyotrophic Lateral Sclerosis. In Yahr, Melvin D. (ed.): Transactions of the American Neurological Association 1964. New York, Springer Publishing Company, Inc., 1964, vol. 89 pp 238-240.

Norris F. H., and Engel, W.K.: Carcinomatous amyotrophic lateral sclerosis in The Remote Effects of Cancer on the Nervous System, Norris, F.H. (ed.), New York, N.Y., Grune & Stratton. In press.

Honors and Awards relating to this project: None.

Serial No. NDB(I)-63 MN/OC 1038 (c)
1. Medical Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Data Analysis in Neuromuscular Disease

Principal Investigators: W. King Engel, M.D.
L.A.H. Hogenhuis, M.D.

Other Investigators: None

This project incorporated in No. 1039

Serial No. NDB(I)-62 MN/OC 918 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Enzymatic Histo- and Cytochemical Studies of the Spinal Cord and Brain in Monkey and Man

Principal Investigators: W. King Engel, M.D.
Jacob C. Jacob, M.D.

Other Investigators: Walter A. Camp, M.D.

This project incorporated in No. 1039

Serial No. NDB(I)-61 MN/OC 804 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Amyotrophic Lateral Sclerosis and Other Lower Motor Neuron Diseases

Principal Investigators: W. King Engel, M.D.
L.A.H. Hogenhuis, M.D.

Other Investigators: William J. Collis, M.D.
Dale E. McFarlin, M.D.

This project incorporated in No. 1039

Serial No. NDB(I)-63 MN/OC 1037 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Microbiology Applied to the Study of Neurologic Disease

Principal Investigator: W. King Engel, M.D.

Other Investigators:

Cooperating Units: None

Man Years:
Total: .1
Professional: .1
Other:

Project Description:

Objectives: To study the growth characteristics, histochemical reactivities, immunologic properties, and electron microscopic details of skeletal muscle grown in tissue culture. The muscle is either chick embryo or normal or abnormal biopsied human muscle.

Methods Employed: Standard tissue culture techniques are used as a basis, with modifications as necessary to obtain good growth from human skeletal muscle. The techniques applied to the material grown are described in other reports.

Patient Material: A portion of the muscle biopsy from certain patients with neuromuscular disease is taken for culture purposes.

Major Findings: Histochemical and cytochemical studies of newly grown chick embryo skeletal muscle fibers show that they contain abundant amounts of the following materials: phosphorylase, glycogen, myosin ATPase, cytochrome oxidase, succinate dehydrogenase, lactate dehydrogenase, menodione-mediated alpha-glycerophosphate dehydrogenase, and DPNH and TPNH diaphorases. The subcellular localization of the colored end-product of these reactions is like that in biopsied chick muscle,

indicating a high degree of muscle fiber differentiation in vitro even though nerve supply is not present.

Preliminary studies show the same techniques are also applicable to spinal cord motor neurons grown in vitro.

Moderate growth of biopsied normal and abnormal human biopsies has been achieved.

Significance to Neurologic Research: In addition to determining the histochemical properties of normal muscle and its growth characteristics in vitro, the studies of abnormal muscle will be used to seek abnormal growth patterns and, more particularly, to determine ways of improving deficient growth. Agents found to improve deficient growth in vitro will then be tested for their usefulness in the patient to promote growth of abnormal muscle or to retard its degeneration. As a foundation for understanding phenomena in human muscle, chick embryo material has been studied. Study of motor neurons grown in vitro in part of a multilateral approach to diseases which preferentially involve human motor neurons.

Proposed Course of Project: Further studies of normal and abnormal human muscle in vitro will be done. Definitive studies, starting with histochemistry, will be applied to the cultured motor neurons.

Part B included No

Serial No. NDB(I)-63 MN/OC 1034 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Myopathies

Principal Investigator: W. King Engel, M.D.

Other Investigators: Leon A.H. Hogenhuis, M.D.
E. Arnold Carmichael, M.D.
Walter A. Camp, M.D.

Cooperating Units: None

Man Years:
Total: 1.7
Professional: 1.7
Other:

Project Description:

Objectives: To analyse patients with all sorts of myopathies in order to test a newly developed classification and to more fully elaborate the various clinical, histochemical, biochemical, and ultrastructural abnormalities of these patients. To treat by various methods to learn which are most effective with each disease category.

Patient Material: Medical Neurology Branch patients.

Major Findings: A new classification of the myopathies has been developed and is proving to be most useful for investigative purposes - its most important feature is that it does not force one to make a specific diagnosis when evidence for such is incomplete.

A symposium on "Current Concepts of the Myopathies" has been published, by invitation, presenting our views. It is too extensive (125 pages) to be summarized.

Therapeutic agents have been used, especially in the inflammatory myopathies with good results. However, in some

cases the greatest benefit was obtained by reducing the medication the patient was taking; presumably the drugs were producing a toxic effect in such patients. Clinical judgement indicates whether a drug is to be increased or decreased.

Although no new myopathies were discovered this year, it was found in 3 patients with thyrotoxic weakness that there was no myopathy in the usual sense. Instead they had about 30% generalized reduction of fiber diameter and scattered fibers with histochemical changes like atrophy.

The "myopathy" of Cushing's syndrome shows atrophied fibers which retain high phosphorylase activity, making them different from both the normal myopathy and the usual denervation atrophy.

Clinical biochemistry - see other project report.

Tissue culture of myopathic biopsies - see other project report.

Histochemistry of myopathic biopsies - see other project report.

Electron-microscopy of myopathic biopsies - see other project report.

Significance to Neurologic Research: The clinical studies related to therapy will help plan a better treatment program for patients with myopathy. The new classification will aid in the investigation and treatment of these patients. The biochemical, histochemical, tissue culture and electron-microscopic studies are providing more detailed information on the abnormalities occurring in the myopathies - information that is hoped to provide a basis for finding ways of treating and preventing these diseases.

Proposed Course of Project: The studies underway are part of a long-term project which will continue for several years.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Engel, W.K.: (Guest Editor) A Symposium "Current Concepts of the Myopathies" in Clinical Orthopaedics and Related Research, pp. 125, 1965.

Engel, W.K.: A Clinical Approach to the Myopathies, Chapter 2. A Symposium "Current Concepts of the Myopathies" in Clinical Orthopaedics and Related Research, pp. 6-18, 1965.

Engel, W.K. and Camp, W.A.: Myopathies Associated with Other Diseases, Chapter 3. A Symposium "Current Concepts of the Myopathies" in Clinical Orthopaedics and Related Research, pp. 19-33, 1965.

Engel, W.K. and Hogenhuis, L.A.H.: Genetically-Determined Myopathies, Chapter 4. A Symposium "Current Concepts of the Myopathies" in Clinical Orthopaedics and Related Research, pp. 34-62, 1965.

Engel, W.K.: Muscle Biopsy, Chapter 7. A Symposium "Current Concepts of the Myopathies" in Clinical Orthopaedics and Related Research, pp. 80-105, 1965.

Engel, W.K., McFarlin, D.E., Drews, G. and Wochner, R.D.: Protein abnormalities in neuromuscular disease. Section on Clinical Science, J.A.M.A. (In Press).

Honors and Awards relating to this project: None.

Serial No. NDB(I)-62 MN/OC 917 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Biochemistry Applied to the Study of Neurologic Disease.

Principal Investigators: W. King Engel, M.D.
Genevieve Drews, M.D.
Leon A.H. Hogenhuis, M.D.
Richard J. Kossmann, M.D.
Irwin Brody, M.D.
Jerome S. Resnick, M.D.

Other Investigators: Samuel H. Boyer, IV, M.D.
Thomas Waldman, M.D.
Raymond D. Wochner, M.D.

Cooperating Units: The Johns Hopkins Hospital, NCI

Man Years:
Total: 1.9
Professional: 1.9
Other:

Project Description:

Objectives: To seek and analyse biochemical abnormalities of neurologic disease.

Methods Employed: Serum and muscle biopsy extracts have been studies with a variety of techniques:

a. myoglobin - starch gel electrophoresis; spectrophotometry of acid met-, alkaline met-, and alkaline cyan- states; partial fingerprinting by paper electrophoresis combined with ascending paper chromatography.

b. lactate dehydrogenase (LDH) isozymes - starch gel electrophoresis, preferential inhibitors, histochemical reaction mix.

c. esterase isozymes - starch gel electrophoresis, "selective" inhibitors, histochemical reaction mix.

d. total body potassium - whole body counter of natural isotope K^{40}.

e. other techniques set up - phosphorylase, glycogen, amylo 1,4 → 1,6 transglucosidase, lactate, and pyruvate.

f. turnover of various serum proteins by following the fate isotopically labelled pure fractions of serum proteins from normal and abnormal persons.

Patient Material: Patients with neuromuscular disease.

Major Findings: Contrary to studies by others no abnormalities of myoglobin were found in several types of muscular dystrophy, nor in paroxysmal rhabdomyolysis (myoglobinuria).

Contrary to the implication of others, the preferential reduction of the LDH-5 isozyme in muscle tissue is not indicative of Duchenne dystrophy, but is found in a variety of neuromuscular diseases, i.e., diseases of muscle as well as diseases secondary to nerve involvement, inherited as well as sporadic conditions, infants and adults. At most, LDH-5 loss may be a sign of abnormal muscle.

Preferential inhibitors of certain LDH isozymes have been described in starch gel electrophoresis. Excess lactate inhibits the slow moving isozymes and urea the fast-moving ones. These inhibitors have been applied to histochemistry and the relative amounts of the various LDH isozymes in subcellular structures have been described; here, too, in the histochemical system LDH-5 is preferentially lost.

In guinea pigs, the LDH isozyme pattern of soleus changes from birth to the adult, and denervation causes reversion to the immature isozyme pattern.

Certain isozymes of serum cholinesterase were selectively absent in a patient with succinylcholine sensitivity.

Total body K^{40} appears to be correlated with muscle mass and not with specific diseases. Carriers of muscular dystrophy did not have an abnormally low total body K^{40}, in contrast with reports of others.

Abnormally short survival of γ-globulin was found in patients with myotonic dystrophy but not in other dystrophies or in ALS.

This hypercatabolism did not affect albumin, or macroglobulin, and may be related especially to the L-chain of γ-globulin.

Significance of Neurologic Research: Elucidation of biochemical abnormalities, particularly in the realm of proteins, is vital to the understanding of neurologic diseases and in seeking means of therapy.

Proposed Course of Project: The above techniques will continue to be applied. Other techniques are also being applied, namely ones for phosphorylase, glycogen, amylo 1,4 → 1,6 transglucosidase, pyruvate, and lactate. Correlation of amounts of phosphorylase detected by histochemistry with biochemical assay will be done. Tolerance tests - namely pyruvate, and arginine - said to be abnormal in amyotrophic lateral sclerosis (ALS) are being applied to ALS patients and various control patients (see ALS project). Further studies of serum and tissue protein metabolism are underway in various neurologic diseases.

Lack of adequate space and a biochemist seriously impair this project.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Brody, I.A.: Effect of denervation on the lactate dehydrogenase isozymes of skeletal muscle. Nature 205: 196, Jan. 1965.

Brody, I.A.: Clinical Biochemistry of the Myopathies, Chapter 6. A Symposium "Current Concepts of the Myopathies" in Clinical Orthopaedics and Related Research, pp. 74-79, 1965.

Brody, I.A.: The significance of lactate dehydrogenase isozymes in abnormal human skeletal muscle. Neurology 14: 1091-1100, Dec. 1964.

Brody, I.A. and Engel, W.K.: Isoenzyme histochemistry: The display of selective lactate dehydrogenase isoenzymes in sections of skeletal muscle. J. Histochem. & Cytochem. 12: 687-695, Sep. 1964.

Brody, I.A., Resnick, J.S., and Engel, W.K.: Detection of atypical serum cholinesterase by electrophoresis. Arch. Neurol. (In Press).

Drews, Genevieve A.: Pharmacological Treatment of Human Myopathies, Chapter 8. A Symposium "Current Concepts of the Myopathies" in Clinical Orthopaedics and Related Research, pp. 106-112, 1965.

Kossmann, R.J., Fainer, D.F., and Boyer, S.H.: A study of myoglobin in disease with comments concerning the myoglobin minor components. Cold Spring Harbor Symposia on Quantitative Biology 29: 375-385, 1964.

Kossmann, R.J., Peterson, D.C., and Andrews, H.L.: Studies in neuromuscular disease. I. Total body potassium in muscular dystrophy and related diseases. Neurology (In Press)

Vates, T.S., Jr., Bonting, S.L., and Oppelt, W.W.: Na-K activated adenosine triphosphatase and formation of cerebrospinal fluid in the cat. Amer. J. Physiol. 206: 1165-1172, May 1964.

Vessell, E.S., and Brody, I.A.: Biological applications of lactic dehydrogenase isozymes: Certain Methodological considerations. Ann. N.Y. Acad. Sci. 121: 544-559, 1964.

Honors and Awards relating to this project: None.

Serial No. NDB(I) -63 MN/OC 1041 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Myoglobin Studies in Neuromuscular Diseases

Principal Investigator: Richard J. Kossmann, M.D.

Other Investigators: Samuel H. Boyer, IV, M.D.

This project incorporated in No. 917

Serial No. NDB(I)-63 MN/OC 1040 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Studies of Body Potassium in Neuromuscular Diseases

Principal Investigator: Richard J. Kossmann, M.D.

Other Investigators: Howard L. Andrews, M.D.

This project incorporated in No. 917

Serial No. NDB(I)-63 MN/OC 1035 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Isoenzymes in Neuromuscular Disease

Principal Investigators: Irwin A. Brody, M.D.
W.King Engel, M.D.

Other Investigators: None

This project incorporated in No. 917

Serial No. NDB(I)-62 MN/OC 915 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Histochemistry Applied to the Study of Neurologic Disease

Principal Investigator: W. King Engel, M.D.

Other Investigators: Irwin A. Brody, M.D.
Michael H. Brooke, M.D.
Dale E. McFarlin, M.D.
Jacob C. Jacob, M.D.
Hanna Jedrejowska, M.D.
Leon A.H. Hogenhuis, M.D.
Genevieve Drews, M.D.

Cooperating Units: None

Man Years:

Total:	5.0
Professional:	3,0
Other:	2,0

Project Description:

Objectives: To study the cellular and subcellular localization of a variety of histochemical reactions in normal human skeletal muscle and neurons and to see how they are altered in neurologic diseases.

Methods Employed: Histochemical techniques for the following reactions are being done on muscle and nerve tissue - they have been outlined in previous reports. Recently added ones include reactions for acid phosphatase (a lysosomal enzyme), esterase, neutral lipid, and EDTA-activated myofibrillar ATPase.

Patient Material: Medical Neurology Branch patients.

Major Findings: Nitro-blue tetrazolium (NBT) is the most commonly used indicator of dehydrogenase activity. However, it has been found to have a heretofore unrecognized selective binding to lipoprotein membranes (mitochondria and sarcoplasmic

reticulum triads) and thereby exert both false negative and false positive localization artifacts.

Phenazine methosulfate (PMS), a commonly used facilitator of electron-transport, has been found to exert a selective "shunt" action bypassing NBT in certain types of muscle fibers and therefore cause false negative localization of activity. Further, this phenazine shunt action is dependent on the relative concentrations of the reagents used, in, for example, the lactate dehydrogenase and DPN-linked alpha-glycerophosphate dehydrogenase reactions.

Mitochondrial aggregates were described as regions of muscle fibers containing high concentration of mitochondria but lacking certain of the mitochondrial enzymes. They are restricted to type II muscle fibers.

The detailed histochemical changes of denervated adult guinea pig muscle were recorded.

Analysis was nearly completed of denervated muscle in newborn guinea pigs.

Subcellular localization of histochemical reactions has been further detailed.

Reviews of our total experience in the histochemistry of muscle biopsies in neuromuscular disease has been published, by request, in a text and in two symposia. The details contained therein are too numerous to be summarized here.

Histochemical analysis of 30 patients with Myasthenia Gravis is discussed under that project.

A new method of study of EDTA-activated myofibrillar ATPase has been devised and its localization studied.

Certain abnormalities in various neuromuscular diseases have been discovered to show preferential involvement of one histochemical fiber type.

A new sort of muscle fiber abnormality has been named "type-II fiber atrophy" and its occurrence described.

Correlation of light-microscopic cytochemistry with electron-microscopy in the study of neurons and muscle cells in patients with neurologic diseases is described in the electron-microscopy project.

Neuron studies - see ALS project.

Significance to Neurologic Research: Histochemistry of skeletal muscle in neuromuscular disease is still in its infancy, and the present work represents the initial cataloging of the various abnormalities. In such unexplored territory it is not surprising that virtually every finding is new.

Of particular interest are the findings of an apparently selective involvement of one histochemical type of muscle fiber, suggesting that the particular characteristics of metabolism of that type of muscle fiber are more susceptible to a given disease process. This permits us to ask why they are more susceptible and conversely, what makes the others more resistant?

Neuron studies - see ALS project.

Proposed Course of Project: The detailed results of histochemical and cytochemical evaluation of muscle biopsies from more than 1000 patients, averaging 10 different histochemical reactions each, are being compiled into an Atlas, due to be completed in about a year.

Additional reactions are being added to the battery of techniques applied to each biopsy.

The basic mechanisms and false localizing factors of the reactions used are being analysed.

Further correlations between light-microscopic cytochemistry and electron-microscopy will be made in muscle and neuron biopsies in human neurologic disease.

We are also beginning to study biopsies from a large number of normal persons. This is necessary because our techniques of cytochemistry and electron-microscopy are showing very subtle changes, which may or may not be found in otherwise normal persons. The diagnostic importance of knowing what is "normal" cannot be underestimated.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Brody, I.A. and Engel, W.K.: Effects of phenazine methosulfate in histochemistry. J. Histochem. & Cytochem. 12: 928-929, 1964.

Hogenhuis, L.A.H., and Engel, W.K.: Histochemistry and cytochemistry of experimentally denervated guinea pig muscle. I. Histochemistry. Acta Anat. 60: 39-65, 1965.

Engel, W.K., and Brooke, M.H.: Muscle biopsy as a clinical diagnositic aid. In Recent Advances in Neurological Diagnostic Methods, (ed.) W.S. Fields, Springfield, Ill., Charles C Thomas, 1965

Engel, W.K.: Diseases of the neuromuscular junction and muscle. In Adams, C. (ed.): Histochemistry and Cytochemistry of the Nervous System. Amsterdam, Elsevier Press, 1965.

Brooke, M.H. and Engel, W.K.: The selective binding of nitro blue tetrazolium. J. Histochem. & Cytochem. (In Press)

Honors and Awards relating to this project: None.

Serial No. NDB(I)-62 MN/OC 920 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Histo- and Cytochemistry of Experimental Animal Neuromuscular Disease in Clinically Oriented Studies

Principal Investigators: Leon A.H. Hogenhuis, M.D.
W. King Engel, M.D.

Other Investigators: Richard L. Irwin, Ph.D.

This project incorporated in No. 915

Serial No. NDB(I)-62 MN/OC 916 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Histochemical Studies on Muscle Specimens from Infantile Spinal Muscular Atrophy

Principal Investigators: Gerald M. Fenichel, M.D.
W. King Engel, M.D.

Other Investigators: None

This project incorporated in No. 915.

Serial No. NDB(I)-62 MN/OC 805 (c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Enzymatic Histo- and Cytochemical Studies of Normal Human Skeletal Muscle.

Principal Investigator: W. King Engel, M.D.

Other Investigators: None

This project incorporated in No. 915

Serial No. NDB(I)-62 MN/OC 914(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Infantile and Juvenile Neuromuscular Disease (including Floppy Babies)

Principal Investigators: W. King Engel, M.D.
T. Wanko, M.D.

Other Investigators: G. Milton Shy, M.D.
G.M. Fenichel, M.D.

This project is completed

Part B included Yes

Serial No. NDB(I)-62 MN/OC 914(c)

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Engel, W.K., Wanko, T., and Fenichel, G.M.:
Nemaline myopathy - a second case. Arch. Neurol.
11: 22-39, Jul. 1964.

Honors and Awards relating to this project: None.

Serial No.NDB(I)-65 MN/NR 1201(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: A Monostapler for use in Vascular Surgery

Principal Investigator: Arthur S. Grove, Jr.

Other Investigator: George Lawrence

Cooperating Units: Instrument Engineering and Development Branch, Clinical Center, NIH

Section on Primate Neurology, NINDB, Clinical Center, NIH

Man Years (computed for the 12 month period)
Total: .1
Professional: .1
Other: 0

Project Description:

Objectives: Stapling devices have been available for years as aids in surgery. Their common use, however, has never been frequent or widespread. Most of the recently developed instruments can only be used for circumferential anastomosis of blood vessels. An instrument for longitudinal approximation of the walls of vessels or grafts would be useful.

Methods Employed: Design, construction and testing of a new type device.

Major Findings: A simple and efficient monostapling device has already been constructed. Preliminary testings of this instrument are being carried out using Teflon tubings.

Significance to Bio-Medical Research and the Program of the Institute: It is hoped that this monostapler will provide an efficient device for closing vascular defects, forming tubular fascial grafts and possibly assisting in vascular anastomoses.

Proposed Course of Project: The largest amount of testing of the stapler will be done in the field of experimental vascular surgery.

Part B included: No

Serial No. NDB(I)-65 MN/NR 1203(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: A Bilateral Angiographic Computer Evaluation of the Superficial Veins and Sinuses of the Brain

Principal Investigators: Giovanni Di Chiro
Norman E. Chase
Irvin I. Kricheff

Other Investigators: None

Cooperating Units: New York University Medical Center, University Hospital, New York, N. Y.

Man Years (computed for the 12 month period)
Total: .1
Professional: .1
Other: 0

Project Description:

Objectives: It has been previously found (Di Chiro) that the angiographic superficial venous hemispheric patterns are often different in the two hemispheres of the same patient. A tentative correlation of these differences was established with functional lateralized cerebral dominance. More specifically, it has been found that the vein of Labbé "predominates," i.e., is larger than other superficial veins in the dominant hemisphere, and the vein of Trolard "predominates" in the nondominant hemisphere with a statistically significant incidence. Considering the implications of these findings it has been decided to check them on a much larger material in order to ameliorate their statistical value.

Methods Employed: The superficial venous drainage is evaluated in the venous phase of cerebral angiography and the "predominant" superficial vein in each hemisphere (Labbé, Trolard or superficial sylvian) is noted. Cases in which bilateral carotid angiography has been carried out are preferred. The handedness and the result of the Wada's test

(if available) are also noted. These data are then fed into a computer for further evaluation.

Major Findings: A specific computer program has been designed for this project and the above described data have already been gathered on many hundreds cerebral angiograms. These data are ready to be fed into the computer.

Significance to Bio-Medical Research and the Program of the Institute: The different angiographic pattern of the superficial discharging veins of the brain and its relationship to the cerebral dominance will, if confirmed, be extremely important from a theoretical point of view. Definite evidence of morphological differences between the two hemispheres would then be available. From a practical point of view, if a good statistical significance of the differences between the two hemispheres (dominant and non-dominant) is found, a new diagnostic method to establish the cerebral functional dominance, would be available.

Proposed Course of Project: To collect further data for computer evaluation.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Di Chiro, G.: Angiographic Patterns of Cerebral Convexity Veins and Superficial Dural Sinuses. Am. J. Roentgenol., Rad. Therapy & Nuclear Med. 87: 308-321, Feb. 1962.

Honors and Awards relating to this project: None

Serial No.NDB(I)-65 MN/NR 1202 (c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Salivary Gland Scanning with Technetium ^{99m} Pertechnetate (Tc^{99m})

Principal Investigators: Giovanni Di Chiro
Arthur S. Grove, Jr.

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: .1
Professional: .1
Other: 0

Project Description:

Objectives: Sialography is the most commonly used radiologic method to evaluate the salivary ducts and glands. Technetium ^{99m} pertechnetate is known to be taken up rapidly and highly specifically by salivary gland tissue. The glands may, therefore, be visualized and their morphology studied by external scanning after intravenous injection of technetium pertechnetate. The aim of this project is to evaluate salivary gland scanning with technetium ^{99m} pertechnetate as a diagnostic technique.

Methods Employed: Patients with normal salivary glands (mostly brain tumor suspects) and patients with salivary tumors and other salivary gland pathology will be studied by external scanning. Most of the patients with salivary pathology will also be subjected to sialography. A comparative study will thus be possible between radiographic and isotopic methods.

Major Findings: A study of normal and pathologic salivary gland morphology in technetium pertechnetate scans is under way.

Significance to Bio-Medical Research and the Program of the Institute: It is hoped that technetium ^{99m} pertechnetate scanning will provide a simple technique for evaluating the presence and nature of lesions in the salivary glands.

Proposed Course of Project: Correlations will be made between sialography and technetium scanning. Results of technetium scans will be compared with clinical pathological findings.

Part B included: No

Serial No. NDB(I)-65 MN/NR 1200
1. Medical Neurology
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Secondary Radiation from Irradiated Residual X-ray Opaque Material in the Spinal Canal.

Principal Investigators: Giovanni Di Chiro
Arthur S. Grove, Jr.

Other Investigators: Emmett Murphy
David Janes

Cooperating Units: Division of Radiological Health, Rockville, Maryland

Man Years (computed for the 12 month period)
Total: .1
Professional: .1
Other: 0

Project Description:

Objectives: Secondary radiation is know to be produced when any material is irradiated with X- or gamma rays. The nature of this secondary radiation is dependent upon the energy of the primary radiation and upon the atomic number of the material irradiated. Previous studies have suggested that beta rays are the most significant secondary radiations produced by irradiation of relatively high atomic number materials such as iodine.

Following myelography, x-ray opaque materials are frequently left behind in the subarachnoid space of the spinal canal. Adhesions may be caused by surgery or by inflammatory response to the contrast materials. The radio opaque media frequently loculate within these adhesions. Therapeutic or repeated diagnostic x-ray studies in this area may produce secondary radiation which would penetrate that segment of the spinal cord lying beneath the contrast material. It is important that we study the nature and amount of this secondary radiation in order to prevent inadvertent damage to the underlying neural tissue.

Methods Employed: After evaluation of a wide variety of solid-state radiation detection devices, we concluded that lithium fluoride (LiF) crystals will be most effective for our use. Radiation absorbed by these crystals can be determined by thermoluminescent techniques. Following irradiation of the crystals with and without an adjacent layer of x-ray opaque contrast media, the secondary radiation produced by the contrast material will be studied. The penetration and type of radiation will be determined by interposing various thicknesses of shielding between the contrast materials and the crystals.

Major Findings: No results have yet been obtained using this technique. The time spent upon this project initially was used for evaluation and selection of the dosimetry devices.

Significance to Bio-Medical Research and the Program of the Institute: Since many patients with retained contrast media require radiation therapy and are exposed to the hazards of secondary radiation, these studies may bring about a new awareness of this problem to many physicians. We believe that the secondary radiation provided inadvertently may be significant and should be calculated for these patients.

Proposed Course of Project: Initially we plan to study secondary radiation from x-ray opaque contrast media by using the techniques described above. In the future, we may extend our studies to phantoms of the spinal canal and possibly to cadavers using lithium fluoride or other solid-state detecting devices.

Part B included: No

Serial No. NDB(I)-65 MN/NR 1199(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Experimental Brain Tumors as a Model for Testing Isotopes used in Scanning

Principal Investigators: Giovanni Di Chiro
Arthur S. Grove, Jr.
Giancarlo Rabotti

Other Investigators: William B. Matthews, Jr.
William F. Bale
Irving Spar
Wirtley R. Anderson
Richard Sellers

Cooperating Units: Laboratory of Viral Carciogenesis, NCI, Clinical Center, NIH

Section on Primate Neurology, NINDB, Clinical Center, NIH

Department of Radiation Biology, University of Rochester, N. Y.

Man Years (computed for the 12 month period)
Total: .9
Professional: .6
Other: .3

Project Description:

Objectives: A large number of radioactive tracers are available for brain scanning. It is practically impossible to evaluate each of these agents in clinical material on a large scale. The aim of this project is to comparatively evaluate in experimental brain tumors, radioactive tracers which are used or usable for brain scanning.

Methods Employed: Multiple brain tumors (gliomas and leptomeningeal sarcomas) have been induced in dogs by intracerebral injection of Rous Sarcoma Virus (RSV). Dogs which

have developed these tumors are intravenously injected with various radioactive tracers. Brain scans are performed at different intervals in order to localize the tumor. The dogs are sacrificed and at autopsy, the sites of the tumors are correlated with the results of the scans. In addition, sample counting of the tumors as well as of normal tissues is carried out in order to evaluate tumoral uptake of the isotopes.

Major Findings: The induction of neoplasia by virus in the brain of large animals (dogs) represents per se a contribution. We have now a biological system to use for evaluation of scanning agents. Brain scanning and sample counting of tumoral and normal tissue of brain tumor bearing dogs have already been started and are being evaluated.

Significance to Bio-Medical Research and the Program of the Institute: Through these experiments we hope to provide an objective and definitive technique for evaluating the usefulness of presently available brain scanning tracers. In addition, we hope to be able to evaluate new tracers which otherwise may not be tested.

Proposed Course of Project: We plan to continue our work using a large series of relatively pure bred dogs in which tumors will be produced. In addition to external brain scanning and sample counting, we contemplate using such techniques as autoradiography, fluorescent radioactive agents and electron microscopy (possibly in autoradiographic material). Emphasis will be placed in the near future upon evaluating the usefulness of fibrinogen, anti-fibrinogen and other comparatively new radioactive-labeled tracers.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Rabotti, G.F., Grove, Jr., A.S., Sellers, R.L. and Anderson, W.R.: Induction of Multiple Brain Tumors (Gliomas and Leptomeningeal Sarcomas) in Dogs by Rous Sarcoma Virus. J. Natl. Cancer Institute (In Press)

Honors and Awards relating to this project: None

Serial No. NDB(I)-65 MN/NR 1198 (c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Brain Scanning (Monograph)

Principal Investigators: Giovanni Di Chiro
Arthur S. Grove, Jr.

Other Investigator: None

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: .2
Professional: .2
Other: 0

Project Description:

Objectives: Continuing progress in radioisotope brain scanning is rapidly making this technique the most frequently used diagnostic tool for demonstrating intracranial lesions. A monograph embracing the various and newest aspects of brain scanning, with an evaluation of the many used and usable tracers, is lacking. The decision to prepare such a monograph was therefore reached.

Methods Employed: Critical bibliographic evaluation and appraisal of our own experience and material.

Major Findings: Organization of the monograph as a whole has been undertaken and a few chapters have been prepared.

Significance to Bio-Medical Research and the Program of the Institute: This monograph will be useful for students and physicians who require an introduction to the subject of brain scanning. In addition, it will be a comprehensive reference work for the investigators using neuroradiologic techniques involving radioisotopes.

Proposed Course of Project: We plan to complete the manuscript of this monograph by July 1966.

Part B included: No

Serial No. NDB(I)-65 MN/NR 1197 (c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Radio-Isotope Brain Scanning for Evaluating the Function of Cerebrospinal Fluid Shunts

Principal Investigators: Giovanni Di Chiro
Arthur S. Grove, Jr.

Other Investigator: William B. Matthews, Jr.

Cooperating Units: Department of Neurosurgery, George Washington University Hospital, Washington, D. C.

Department of Neurosurgery, Walter Reed Medical Center, Washington, D. C.

Man Years (computed for the 12 month period)
Total: .2
Professional: .2
Other: 0

Project Description:

Objectives: A wide variety of procedures have been used for shunting cerebrospinal fluid in the treatment of hydrocephalus and increased intracranial pressure. Following the establishment of a cerebrospinal fluid shunt, the problem frequently arises of whether the tube is patent and is functioning properly. Several tests are available to detect and to locate a block if it exists. Most of these tests, however, are clinically inadequate. The object of this study is to develop a new procedure for evaluating the function of cerebrospinal fluid shunts using radioactive isotopes and brain scanning.

Methods Employed: A small volume of either I^{131}-labeled serum albumin or technetium ^{99m} pertechnetate is injected into the shunt tubing using a small gauge needle, provided that the tubing is made of a suitable material. Soon after injection, four-view brain scans are carried out using our Tetrascanner (see Project NDB(I)-63 MN/NR 1047(c). The shunt tubing itself

is frequently outlined by this technique. If the shunt is patent and is functioning properly, the ventricles and cisterna magna are also visualized (in the case of ventriculo-cisternal shunts). In ventriculo-jugular shunts, patency of the distal end is proven by rapid uptake of technetium by the salivary glands, for which technetium pertechnetate has a marked affinity.

Major Findings: It has been shown that the function and patency of cerebrospinal fluid shunts can be evaluated by using radio-isotopes and brain scanning.

Significance to Bio-Medical Research and the Program of the Institute: This technique quickly and safely demonstrates the patency and evaluates the function of cerebrospinal fluid shunts. It is the first technique which permits the study of the gross morphology of these shunts and the communicating cerebrospinal fluid system.

Proposed Course of Project: We plan to continue a co-operative effort with the Department of Neurosurgery of the George Washington University Hospital and Walter Reed Medical Center in evaluating additional patients and in accumulating more experience with this technique.

Part B included: No

Serial No. NDB(I)-65 MN/NR 1196(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Clinical Comparison of Radio-Iodinated Serum Albumin and Technetium ^{99m} Pertechnetate as Brain Scanning Agents

Principal Investigator: Giovanni Di Chiro

Other Investigator: William B. Matthews, Jr.

Cooperating Units: Radiation Safety Department, Clinical Center, NIH

Radiopharmaceutical Service, Pharmacy Department, Clinical Center, NIH

Man Years (computed for the 12 month period)
Total: .4
Professional: .1
Other: .3

Project Description:

Objectives: Evaluations of the relative merits of various isotopes used for brain scanning (RISA, Hg^{203}, Hg^{197}, As^{74} and TC^{99m}) have recently been published. These studies have all been conducted investigating different isotopes in different groups of patients. This type of comparative study is of necessity only of relative value since it is practically impossible to obtain comparable groups of patients with intracranial space occupying lesions. On the other hand, multiple scans with multiple isotopes carried out in the same patients, in our opinion, lend themselves much better to a comparative appraisal of the various scanning agents. The physical characteristics of RISA and technetium ^{99m} pertechnetate make these two agents paticularly suitable for use in a comparative study with double isotope-double scan in the same patient.

Methods Employed: Each patient is injected with one millicurie (1 mc) of technetium 99m pertechnetate and a brain scan is carried out about one hour after injection. Immediately

after this scan the patient receives the usual doses of RISA. The day after, the technetium activity having decayed, a new scan is carried out recording the I^{131} activity. The two scans are then compared keeping in mind the different normal distribution patterns of the tracers. The radioactive dosimetry presents no problem, because one millicurie (1 mc) of technetium ^{99m} pertechnetate increases the total body radiation to the patient only one-hundredth of the dosage administered with RISA.

Major Findings: Over 80 patients have been injected up to the present time with double isotope-double scan technique using RISA and technetium ^{99m} pertechnetate. It is already clear that in selected cases only one of the two compounds will concentrate in the tumoral lesion to such a degree as to make a diagnostic scan possible. In a larger group of patients, the uptake of one of the two compounds is better or much better, although a diagnostic scan is also obtained with the other compound.

Significance to Bio-Medical Research and the Program of the Institute: This study represents a contribution to the problem of which radioactive tracer is better for brain scanning. In addition, with this study, some information will be obtained on the problem of selective uptake of specific radioactive drugs by the various types of tumors.

Proposed Course of Project: To gather further clinical material in which the double isotope-double scan technique is carried out. To appraise the double scans already available and those available in the near future.

Part B included: No

Serial No. NDB(I)-65 MN/NR 1195(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Subtraction-Angiography in Spinal Cord Lesions

Principal Investigators: Giovanni Di Chiro
John L. Doppman

Other Investigators: None

Cooperating Units: Diagnostic X-Ray Department, Clinical Center, NIH

Man Years (computed for the 12 month period)
Total: .1
Professional: .1
Other: 0

Project Description:

Objectives: Diagnosis of the nature of space occupying lesions within the spinal canal is often difficult. In most instances with myelography, we are only able to put in evidence a total or partial subarachnoidal block. Myelograms, on the other hand, are seldom helpful in establishing the type of lesion which has caused the block. With recent advances in the field of arteriography - safer radiographic contrast media, better needles and better catheters, and the revival of interest in subtraction - we feel that a systematic angiographic evaluation of the intraspinal mass lesions would be very worthwhile. Angiography is of particular importance, as has already been proven by French authors, in spinal cord vascular malformations. Significant information might, however, be obtained in other types of lesions, especially spinal cord tumors. It is conceivable that tumor stains, and pathologic vessels may be demonstrated in cases of spinal cord tumors in analogy to similar angiographic findings in brain tumors.

Methods Employed: Selective arteriograms with the use of modern catheter techniques are carried out in patients in whom intra-spinal space occupying lesions are suspected. The subtraction technique of Ziedses des Plantes is used to better

visualize the injected vessels.

Major Findings: Valuable diagnostic information has been obtained in isolated cases of spinal cord vascular malformations with mapping of the feeding arteries and the discharging veins.

Significance to Bio-Medical Research and the Program of the Institute: It is possible that angiography of the spinal cord will acquire in the future the same importance of cerebral angiography.

Proposed Course of Project: To gather clinical-angiographic material in patients with spinal cord disease.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Doppman, J.L. and Di Chiro, G.: Subtraction-Angiography of Spinal Cord Vascular Malformations: Report of a case. J. Neurosurg. (In Press)

Honors and Awards relating to this project: None

Serial No. NDB(I)-63 MN/NR 1048(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Prognostic Significance of Parasellar Carotid Calcifications

Principal Investigators: Giovanni Di Chiro
Leslie Libow

Other Investigators: None

Cooperating Units: Section on Cerebral Metabolism, Laboratory of Clinical Science, NIMH

Man Years (computed for the 12 month period)
Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: Same as NDB(I)-63 MN/NR 1048(c)

Methods Employed: Same as NDB(I)-63 MN/NR 1048(c)

Major Findings: Same as NDB(I)-63 MN/NR 1048(c)

Significance to Bio-Medical Research and the Program of the Institute: Same as NDB(I)-63 MN/NR 1048(c)

Proposed Course of Project: The followup of the particular group of patients used for this project will be completed at the end of 1966 - ten years after the beginning of clinical evaluation. At that time it is hoped that the prognostic appraisal of the parasellar carotid calcifications will be established.

Part B included No

Serial No. NDB(I)-63 MN/NR 1047(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Isotope-Ventriculography and Isotope-Cisternography

Principal Investigator: Giovanni Di Chiro

Other Investigator: William B. Matthews, Jr.

Cooperating Units: Radiation Safety Department, Clinical Center, NIH

Radiopharmaceutical Service, Pharmacy Department, Clinical Center, NIH

Man Years (computed for the 12 month period)
Total: .5
Professional: .1
Other: .4

Project Description:

Objectives: Same as NDB(I)-63 MN/NR 1047(c)

Methods Employed: As in the previous report except for the addition of the Tetrascanner.

In recent months, the brain scans have been carried out with a new unit, the Tetrascanner. This detecting unit is provided with four probes which scan the patient's head simultaneously from four sources. Antero-posterior, postero-anterior, right and left lateral head scans are thus obtained at the same time. This type of recording allows the simultaneous three-dimensional study of the movement of the cerebrospinal fluid.

Major Findings: Isotope-ventriculography and isotope-cisternography have given us a clearer concept of the cerebrospinal fluid dynamics in human beings. It has now been proven beyond any doubt that at least a part of the cerebrospinal fluid moves in a flow-like fashion toward the hemispheric convexities, possibly due to the vascular and

choroid plexus pulsations, plus the vis a tergo of the newly produced fluid.

In the pathological field, isotope-ventriculography and isotope-cisternography have proven to be extremely valuable diagnostic tools. Isotope-cisternography is the best available diagnostic test for the localization of cerebrospinal fluid rhinorrhea and otorrhea. Isotope-cisternography is the first test to allow us to study objectively that vast terra incognita represented by the subarachnoid spaces from the basal cisterns up to the superior longitudinal sinus. Pneumoencephalography is unreliable in the evaluation of the convexity subarachnoidal pathways. In many instances, unsuspected pathology of the convexity subarachnoidal spaces has been established by isotope-cisternography. Such instances include subarachnoidal blocks at various levels and of various types, leptomeningeal cysts and spontaneous ventriculostomies. One finding, very important from the theoretical point of view, has been that whenever a block of the convexity subarachnoidal spaces exists, the fluid "back-flows" into the usually dilated ventricular system. This "back-flow", which never occurs in normal cases, may be interpreted as a proof that in such pathological cases the choroid plexuses function as vicarious resorbing agents.

Significance to Bio-Medical Research and the Program of the Institute: Same as NDB(I)-63 MN/NR 1047(c)

Proposed Course of Project: With the help of the Tetrascanner and with the experience already acquired, the mapping and timing of the cerebrospinal fluid flow will be subjected to further detailed investigation. Particular emphasis will be put upon the pathological aspects.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Di Chiro, G.: New Radiographic and Isotopic Procedures in Neurological Diagnosis. JAMA 188: 524-529, May 1964.

Di Chiro, G.: Anatomical, Three-Dimensional Brain Scanning. In Planiol, T. (Ed.): Radio-Isotopes et Affections du Systeme Nerveux Central. Paris, France, Masson & Cie, 1964, pp. 35-49.

Di Chiro, G.: Movement of the Cerebrospinal Fluid in Human Beings. Nature 204: 290-291, October 1964.

Di Chiro, G.: New Observations on the Circulation of the Cerebrospinal Fluid. ACTA Radiol. (In Press)

Honors and Awards relating to this project: None

Serial No. NDB(I)-62 MN/NR 925(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Small Sella Turcica

Principal Investigators: Giovanni Di Chiro
Thomas Aceto, Jr.
Mary Parker
Alvin Hayles

Other Investigators: None

Cooperating Units: The National Pituitary Agency, Baltimore, Maryland

The Children's Hospital, Buffalo, New York

Washington University, St. Louis, Missouri

The Mayo Clinic, Rochester, Minnesota

Man Years (computed for the 12 month period)
Total: .1
Professional: .1
Other: 0

Project Description:

Objectives: Same as NDB(I)-62 MN/NR 925(c)

Methods Employed: Same as NDB(I)-62 MN/NR 925(c)

Major Findings: Same as NDB(I)-62 MN/NR 925(c)

Significance to Bio-Medical Research and the Program of the Institute: Same as NDB(I)-62 MN/NR 925(c)

Proposed Course of Project: A collaborative study on the effects of growth hormones in dwarfs is being carried out under the auspices of the National Pituitary Agency. The skull x-rays of the hypopituitary dwarfs included in this project - in whom according to Di Chiro and Fisher findings -

a small sella is frequently found - will all be evaluated by Di Chiro. Emphasis will be placed upon the comparison of repeated sella turcica measurements taken during the followup of the patients treated with growth hormones.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Fisher, R.L. and Di Chiro, G.: The Small Sella Turcica. Am. J. Roentgenol., Rad. Therapy & Nuclear Med. 91: 996-1008, May 1964.

Honors and Awards relating to this project: None

Serial No. NDB(I)-62 MN/NR 922(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: An Atlas of Pathologic Pneumoencephalographic Anatomy

Principal Investigators: Giovanni Di Chiro
Mannie M. Schechter
Ingmar Wickbom

Other Investigators: None

Cooperating Units: Albert Einstein College of Medicine, Yeshiva University, New York, New York

Sahlgrenska Sjukhuset, Gothenburg, Sweden

Man Years (computed for the 12 month period)
Total: .1
Professional: .1
Other: 0

Project Description:

Objectives: Same as NDB(I)-62 MN/NR 922(c)

Methods Employed: Same as NDB(I)-62 MN/NR 922(c)

Major Findings: Same as NDB(I)-62 MN/NR 922(c)

Significance to Bio-Medical Research and the Program of the Institute: Same as NDB(I)-62 MN/NR 922(c)

Proposed Course of Project: This atlas is being prepared for publication in the near future.

Part B included No

Serial No. NDB(I)-62 MN/NR 921(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Jugular Foramen

Principal Investigators: Giovanni Di Chiro
Robert L. Fisher

Other Investigator: Karin B. Nelson

Cooperating Units: Diagnostic X-ray Department, Clinical Center, NIH

Smithsonian Institute, Washington, D.C.

Neurosurgical Clinic, University of Padua, Padua, Italy

Man Years (computed for the 12 month period)
Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: Same as NDB(I)-62 MN/NR 921(c)

Methods Employed: Same as NDB(I)-62 MN/NR 921(c)

Major Findings: Same as NDB(I)-62 MN/NR 921(c)

Significance to Bio-Medical Research and the Program of the Institute: Same as NDB(I)-62 MN/NR 921(c)

Proposed Course of Project: This project is completed.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Di Chiro, G., Fisher, R.L. and Nelson, K.B.: The Jugular Foramen. J. Neurosurg. 21: 447-460, June 1964.

Honors and Awards relating to this project: None

Serial No. NDB(I)-62 MN/NR 924(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Contrast Radiography of the Spinal Cord

Principal Investigators: Giovanni Di Chiro
Robert L. Fisher

Other Investigators: None

Cooperating Units: Pathology Department, Clinical Center, NIH

Diagnostic X-Ray Department, Clinical Center, NIH

X-Ray Department, Washington Hospital Center, Washington, D. C.

X-Ray Department, Walter Reed Army Medical Center, Washington, D. C.

X-Ray Department, Landeskrankenhaus, Graz, Austria

Man Years (computed for the 12 month period)
Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: Same as NDB(I)-62 MN/NR 924(c)

Methods Employed: Same as NDB(I)-62 MN/NR 924(c)

Major Findings: Same as NDB(I)-62 MN/NR 924(c)

Significance to Bio-Medical Research and the Program of the Institute: Same as NDB(I)-62 MN/NR 924(c)

Proposed Course of Project: This project is completed.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Di Chiro, G. and Fisher, R.L.: Contrast Radiography of the Spinal Cord. Archiv. Neurol. 11: 125-143, Aug. 1964.

Honors and Awards relating to this project:

Lewis Clark Wagner Award, Hospital for Special Surgery, New York, New York

Serial No. NDB(I)-62 MN/NR 923(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Clivus

Principal Investigators: Giovanni Di Chiro
William Bruce Anderson

Other Investigators: None

Cooperating Units: Diagnostic X-ray Department, Clinical Center, NIH

Smithsonian Institute, Washington, D. C.

Man Years: (computed for the 12 month period)
Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: Same as NDB(I)-62 MN/NR 923(c)

Methods Employed: Same as NDB(I)-62 MN/NR 923(c)

Major Findings: Same as NDB(I)-62 MN/NR 923(c)

Significance to Bio-Medical Research and the Program of the Institute: Same as NDB(I)-62 MN/NR 923(c)

Proposed Course of Project: This project is completed.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Di Chiro, G. and Anderson, W.B.: The Clivus. Clin. Radiol. (In Press)

Honors and Awards relating to this project: None

Serial No. NDB(I)-61 MN/NR 806(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Axial Transverse Encephalography

Principal Investigator: Giovanni Di Chiro

Other Investigators: None

Cooperating Units: Diagnostic X-Ray Department, Clinical Center, NIH

Man Years (computed for the 12 month period)
Total: .1
Professional: .1
Other: 0

Project Description:

Objectives: Same as NDB(I)-61 MN/NR 806(c)

Methods Employed: Same as NDB(I)-61 MN/NR 806(c)

Major Findings: Axial transverse encephalograms are now taken as routine in the great majority of patients on whom pneumoencephalography is carried out. The axial transverse cuts have offered valuable diagnostic information in many patients with tumors and other intracranial lesions. These have proven to be particularly useful in mass lesions which protrude within the ventricular system.

Significance to Bio-Medical Research and the Program of the Institute: Same as NDB(I)-61 MN/NR 806(c)

Proposed Course of Project: Gathering of experience with this useful technical refinement of pneumoencephalography will be continued.

Part B included Yes No

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Di Chiro, G.: Axial Transverse Encephalography. Amer. J. Roentgen. 92: 441-447, August, 1964.

Di Chiro, G.: New Radiographic and Isotopic Procedures in Neurological Diagnosis. JAMA 188: 524-529, May, 1964.

Di Chiro, G.: Axial Transverse Encephalography with the Radiotome. Medicamundi (In Press)

Honors and Awards relating to this project: None

Serial No. NDB(I)-65 MN/AP 1194 (c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Genetic studies of abnormal plasma cholinesterase in relation to drug metabolism.

Principal Investigator: Richard L. Irwin

Other Investigators: Manfred M. Hein
Jerome S. Resnick
Gerald N. Gold

Cooperating Units: None

Man Years:

Total:	.4
Professional	.4
Other	0

Project Description:

Objective: Soon after the neuromuscular blocking agent succinylcholine began to be used during anesthesia it was observed that many individuals failed to recover promptly from its paralytic effects. The prolongation of paralysis is related to low cholinesterase activity in the plasma which fails to metabolize the succinylcholine to a non-paralytic level. It is known that certain aspects of cholinesterase activity is heritable. The objective of this project is to further define the genetic basis of the occurrence of abnormal cholinesterase in relation to drug metabolism and action.

Methods: Cholinesterase levels, inhibition and substrate specificity of appropriate subjects are studied using titrametric and spectrophotometric techniques. Genetic genotypes are determined by dibucaine and fluoride inhibition tests.

Major Findings: Abnormal plasma esterase activity was found in a family of four in which the mother had previously responded to succinylcholine with a prolonged paralysis. Her plasma failed to metabolize succinylcholine and thus a correla-

tion was established between lack of esterase activity and drug response.

Each of the 4 family members had a unique substrate activity pattern for plasma esterase but only 3 phenotypes were identified. The two siblings had identical phenotypes but markedly different substrate specificity for cholinesterase. The father and son which had the most nearly alike cholinesterase substrate specificity had distinctly different phenotypes. The study indicates that the genetic pattern of inheritance of cholinesterase is not always paralleled by the ability to metabolize drugs or enzyme substrates.

Another family is being studied in which one member had low plasma cholinesterase level accompanied by normal dibucaine inhibition and abnormal fluoride inhibition. The study will continue in order to determine whether the abnormality is genetically based.

A paper entitled "Substrate Specificity and Phenotypes in a Family with Atypical Cholinesterase" has been accepted for publication by the American Journal of Human Genetics.

Significance to the Program of the Institute: The inability of an individual to metabolize succinylcholine endangers life and imposes rigorous procedures upon medical and nursing staffs following surgical procedures upon such individuals. A more complete definition of the problem is therefore desirable in relation to drug use.

Proposed Course of Project: A continuation and extension of the study described herein.

Part B included no

Serial No. NDB(I)-63 MN/AP 1050(C)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The relationship between the functional activity and the mechanical properties of normal and modified striated muscle.

Principal Investigator: Jay B. Wells

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.4
Professional:	.4
Other:	0

Project Description:

Objectives: The determination of the effect of changes in function on the nature of the active contractile component of mammalian limb muscle. Characterization of the series elastic elements with respect to (1) stress-strain relationship and (2) recovery of stored elastic energy during quick release, in muscles modified as described below.

Methods Employed: A small metal appliance has been developed in cooperation with the instrument development section which when aseptically installed in rats immobilized the ankle joint. Length changes were thus prevented in the triceps surae and anterior tibialis muscles. Two to four weeks after application the animals were subjected to the appropriate mechanical procedures leading to the objectives stated above. The method of modification produces changes in the muscle environment while leaving the nerve supply intact. Thus trophic influences due to nerve injury or section were not present.

Major Findings: Joint immobilization in soleus and anterior tibialis muscles produced atrophy, presumed to be from disuse, as shown by comparison of corresponding muscle weights between fixed and normal limbs. Immobilized soleus and anterior

tibialis muscles weighed an average of 56 and 61% respectively of the normal muscle (other limb). Preliminary histological examination of the atrophic muscle showed a reduction in the cross-sectional area of individual muscle fibers without an obvious reduction in the number of fibers. Cell counts would be necessary to establish the latter observation with certainty.

Mechanical responses of the active contractile components of fixed soleus muscle (as represented by the force-velocity relationship) when compared with those of normal muscle, showed a significant increase in the intrinsic shortening velocity (V_o) of the muscle. This constant is defined as the shortening rate of unloaded muscle maximally stimulated and is estimated by the equation V

$$V_o = P_o \frac{b}{a}$$

with the constants P_o a and b derived from the force-velocity relation. The maximal isometric force (P_o) developed by the modified slow muscle was significantly less in comparison with that developed by unaltered muscle. The difference remained when the maximal force was calculated per gram of muscle weight or per cross-sectional area to allow for the atrophy. Thus the constants V_o and P_o derived from immobilized soleus muscle approached the corresponding values calculated for the phasic anterior tibialis muscle.

Significance to the Program of the Institute: The interpretation of results derived in this study should be directly applicable to functional contractile changes which occur in myopathy in which the neuromuscular apparatus is not primarily involved. Moreover, additional information relating to processes of functional adaptation in response to environmental changes will be acquired.

Proposed Course of Project: The possibility of both contractile and elastic elements sharing a common active mechanism suggests an analysis and comparison of the elastic component of modified with unaltered muscle. The phasic anterior tibialis muscle similarly immobilized will be examined for functional changes due to limb fixation. Examination and comparison of the motor nerve activity between fixed and unaltered muscles will permit an evaluation of the influence of nerve activity during production of this atrophy.

Part B included No

Serial No. NDB(I)-63 MN/AP 1049(C)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Mechanical properties of muscle in relation to drug action.

Principal Investigator: Jay B. Wells

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .55
Professional: .55
Other: 0

Project Description:

Objectives: A necessary prerequisite for evaluation of drug effects on tissue responses is an accurate description of the observed response during the drug free or normal state. Therefore, the mechanical properties of the functionally different rat hind limb muscles were reexamined using improved apparatus and techniques with particular emphasis on the series elastic component of the muscles.

Methods Employed: Changes in muscle length and tension development were recorded simultaneously during muscular activity by standard optical and force-displacement transducer apparatus. A unique method of muscle loading was achieved by using constant tension springs which exhibit relatively low inertia, hence provide increased fidelity in registration of muscle displacement.

The apparatus was physically characterized by methods and concepts commonly employed in the field of mechanics. The mechanical characteristics of two rat skeletal muscles have been measured and analyzed with respect to the active contractile component and the series elastic element.

Complete analysis of the results were based on physical

models of muscle established by previous research in this field.

Major Findings: The elastic element in slower contracting soleus muscle, which was "stretched" during full tension development, shortened (when quickly released) at about half the rate of the corresponding response observed in the faster anterior tibialis muscle. The maximal active shortening rate of the soleus contractile element is also about half that of the tibialis muscle. Several different lines of inquiry (in this lab) have shown considerable damping in the elastic recoil of both slow and fast muscle. The similarity in the compared ratio values of elastic to active shortening rates between the two muscle types and evidence of measurable damping in both elastic and contractile retractions strongly support the suggestion of a common origin for these two components of muscle.

The series elastic element in rat hind limb muscle was subject to further examination by using two independent procedures to determine its stress-strain relationship. Curves obtained by the different procedures were not in agreement. Additional investigation required to clarify this apparent variation may yield new insite into the nature of this muscle component. Part of the above findings are included in a paper accepted by The Journal of Physiology (London) for publication. Some of the remaining results comprise a manuscript now in preparation for publication.

Significance to the Program of the Institute: Quantitative description of the mechanical properties of active muscle is necessary for full understanding of the physiological responses of normal and diseased muscle. The present study contributes toward that end and in addition establishes normal values upon which to assess the action of drugs which influence muscular contraction.

Proposed Course of Project: Analysis of elastic retraction under various loads to quantitate the degree of damping shown by the series elastic component. Measurement and analysis of both elastic and contractile responses subjected to the influence of drugs known to act on the muscle membrane.

Part B included No

Serial No. NDB(I)-62 MN/AP 926(C)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Electromechanical coupling in muscle and drug activity.

Principal Investigator: Richard L. Irwin

Other Investigators: Manfred M. Hein

Cooperating Units: None

Man Years:

Total	1.55
Professional:	1.55
Other:	0

Project Description:

Objectives: How the electrical events of muscle membranes by movement of ions induce protein molecules to slide or coil and thus produce shortening or tension has not been experimentally explained. The object of the present study is to explain the ionic basis for the electromechanical coupling of skeletal muscle.

Methods Employed: The general methods used are described in detail in the project report for Fiscal 1964. During Fiscal Year 1965 additional methods have been introduced in order to further study the precise relationships between slow tension development, ionic content and electrical activity. A spectrophotometric method which detects the color changes of a dye in the presence of Ca^{++} has been used to analyze for total muscle calcium and calcium loss. A modified sucrose gap technique has been used to measure changes in electrical potentials in relation to ionic changes within muscle. The use of this method necessitated the development of suitable electrodes for use with rapidly flowing fluids.

Major Findings: Certain smooth muscles have been found to develop contractures in response to Ca^{++} deprivation. The contractures are like those which develop in slow type striated muscles. The anterior bysuss retractor muscle of Mytilus Edulis

(ABRM), a smooth muscle which develops contracture during Ca^{++} lack, contains only one type muscle and each fiber traverses the length of the muscle. This unique geometric property of the ABRM permits its use as a model to study electrical events (by use of extracellular electrodes) in relation to ionic changes. (All known vertebrate muscles which contain slow skeletal muscle also contain fast or twitch type fibers).

The contractures in response to Ca lack, which occur in the ABRM, are followed by relaxation to the original resting tension. The contractures are not neurogenic since they develop in high concentrations of procaine, d-tubocurarine, or atropine. The resting membrane potential does not change as calcium is depleted. Depolarization by KCl after Ca^{++} deprivation is the same as when Ca^{++} is present and repolarization occurs when KCl is replaced without return of Ca^{++}. Transient potentials arise in muscle membranes during calcium withdrawal which are not prevented by procaine, d-tubocurarine, or atropine. The potentials are not temporally related to tension development. Some muscles (ABRM) fail to develop the transient potentials but do develop tension without change in resting potential level. The results thus indicate that tension develops in response to an intracellular movement of Ca^{++} which can occur unrelated to electrical potential change. Under physiological conditions, the electrical potential may accomplish the intracellular movement of calcium. Manuscripts describing these findings are in preparation.

Significance to the Program of the Institute: The present study relates to physiological events in skeletal muscle which are not well understood. It thus may lead to an understanding of muscle function in diseases which affect the skeletal muscles. The occurrence of slow skeletal muscle fibers has recently been identified in the extraocular muscle of mammals. The contractures of extraocular muscles in human beings after administration of depolarizing drugs suggests that slow type muscle fibers exist in the human being although they have not as yet been described. A greater understanding of this slow muscle system, and smooth muscle system with similar responses, is therefore desirable.

Proposed Course of Project: Several other aspects of excitation contracture have been under study during the past year. The results are incomplete and study will continue: (a) membrane effects of Ca^{++} in relation to drug induced contracture; (b) activity of other divalent ions in replacement of Ca^{++}; (c) development of tension in fast type fiber by depletion of external ions; (d) mechanical response of Ca depleted slow striated upon Ca^{++} reloading. The project will continue to furnish data in an attempt to establish the ionic basis for

electromechanical coupling in skeletal muscle.

Part B included No

Serial No. NDB(I)-61 MN/AP 807(C)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Md.
Con't Project-Ser. No. 8a(C) 1961

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: A comparative pharmacological study of fast and slow muscles.

Principal Investigator: Richard L. Irwin

Other Investigators: Jay B. Wells

Cooperating Units: None

Man Years:

Total:	.1
Professional	.1
Other	0

Project Description:

Objectives: Refer to Serial No. NINDB-8a(C) 1962.

Methods Employed: Refer to Serial No. NINDB-8a(C) 1962.

Major Findings: The data collection and conversion system developed under this project has undergone preliminary testing.

The height of isometric tension traces of fast twitch muscles correlated linearly with the area of tension traces.

The distribution of individual tension trace values were found to be widely scattered in relation to the desired precision. This scatter was caused by instability in the strain gauge amplifier.

Significance to the Program of the Institute: Refer to Serial No. NINDB-8a(C) 1962.

Proposed Course of the Project: The necessary instrument modification will be accomplished to correct for amplifier instability and testing for reliability will continue. Data

collected and computer programming will be used to further investigate the pharmacological differences between fast and slow twitch muscles.

Part B included No

ANNUAL REPORT
July 1, 1964 through June 30, 1965
Branch of Surgical Neurology, IR
National Institute of Neurological Diseases and Blindness

Maitland Baldwin, M. D., Chief

During the period of this report, the Branch conducted investigations under the following categorical titles: cerebral trauma, developmental defects, epilepsy, involuntary movements, brain tumor, cerebral edema, language and memory, effects of low temperatures, microbial analysis of neurosurgical environments, and neurosurgical monitoring. Twenty-seven reports were prepared for publication.

As of this date, 22 April 1965, 202 persons participated in the clinical investigations as inpatients, and 443 outpatients were examined in a total of 568 visits. There were 110 major operative procedures.

Branch investigations have been conducted in active collaboration with the following organizations: Branch of Electroencephalography, NINDB; Laboratory of Neurochemistry, NINDB; Laboratory of Neurophysiology, NINDB; Biometrics Branch, NINDB; Clinical Center (Departments of Clinical Pathology and Diagnostic X-ray); Instrument Engineering & Development Branch, DRS, NIH; Computer Facilities, DRS, NIH; Office of Medical Examiner, City of New York; Walter Reed Army Medical Research Institute; Army Medical Research Laboratory, Ft. Knox; Bureau of Standards; Personnel Protection, David Taylor Model Basin, Department of the Navy; U. S. School of Aviation Medicine, Department of the Navy, Pensacola.

Cerebral Trauma

Investigation of the effects of cerebral trauma is continuing in the laboratory and in the clinic. In the laboratory, the nature of the relation between cerebral concussion and the impulse of the blow is being studied. An experimental model of cerebral concussion in the monkey is now being tested under a variety of conditions in order to better understand the mechanics of concussion. The impact force, velocity, linear acceleration of the head, intracranial pressure changes, and head displacement have been measured. Based on this data, a theoretical model for experimental head injury is being developed.

Continuation of radiological studies after carotid angiography revealed that the marked slowing of circulation described in last year's report (within the period of 15-30 minutes after trauma) is of early onset (within 1-2 minutes of the blow) and appears to parallel the development and persistence of post-traumatic cerebral edema.

Clinical studies suggest that persons recovering from the effects of severe deceleration injury without additional complication, display an accelerated recapitulation of postnatal life history. This seems particularly

true where bilateral temporal involvement is a feature of the traumatic lesions.

Developmental Defects

Clinical, biochemical and genetical studies of mental retardation, and progressive cerebral degeneration and cerebral palsy; certain pathological lesions occurring during prenatal, intranatal and early postnatal life; a cytogenetical study of human chromosomes, especially in patients with congenital malformations and mongoloids; cranial measurements in males and females; and a biochemical study of the amino acid analogue, Trifluoroleucine, are subtitles under which problems of developmental defects have been approached during the past year.

The investigation of ten patients with Hurler's syndrome revealed that autosomal recessive and sex-linked varieties cannot be distinguished by the type of mucopolysaccharides excreted. Also, a group of families was identified in which the mode of transmission of the abnormal trait (mental deficiency) was of an autosomal dominant type. Further attention was also paid to the eighth year in the life history of head-joined Siamese twins which have been separated surgically. These appear without discernible functional involvement at this time.

In a patho-anatomical study of developmental porencephalies and polymicrogyria based on eleven human brains, it seems clear that the gross defects involve mesial and parasagittal areas of both hemispheres, as well as large portions of centro-lateral aspects of the hemispheres, while the entire cerebral convexity may be absent. From the histology, it seems that such lesions may originate prior to the sixth month of gestation and may be related to a common causative factor. Analysis of topographical distribution of the large pallial defects and of the polymicrogyria in the present series suggests arterial origin of these two lesions. In another series of patients, karyotypic examination of 122 persons revealed chromosomal abnormalities of diagnostic importance in 19 per cent. Moreover, one set of female twins, one of whom is a mongoloid and has 48 chromosomes, and the other is normal and has 46 chromosomes, was proven to be identical by blood group, protein types and palm prints, despite the discordant trait of mongolism. It is thought that the original zygote was a 47 chromosome mongoloid.

Epilepsy

The clinical and biochemical study of epilepsy in childhood continues and during this period has involved 32 patients. In one group from this series, a l-leucine sensitive hypoglycemia was discovered. This was satisfactorily controlled and the seizures eradicated by the development of a diet which restricts l-leucine and is administered in seven divided portions so as to separate principal feedings of protein and carbohydrates.

The ketogenic diet has been re-evaluated in ten children with generalized seizures. Through the detailed study of their lipid profiles, it was found that the average values for the total lipids, total cholesterol

and total ketones in the plasma increased significantly by the tenth day of the high fat diet, while increase in plasma phospholipids and triglycerides was less striking. Plasma glucose levels were consistently low when the children were on the diet.

Ninety-three patients with epilepsy have been studied in the operating theater during the course of therapeutic procedures designed for the relief of a convulsive disorder. During the course of single unit recording from epileptic cortex of patients, it seemed that two types of cell units were present: those that discharged synchronously with the epileptic waves, and those that discharged independently of the epileptic waves. In selected patients with severe unlocalized (centrencephalic) cerebral seizures, stereotaxically placed depth lesions in the Fields of Forel and the zona incerta have been made, and similarly, lesions have been placed in the amygdala by stereotaxic instrument in certain cases of poorly lateralized cerebral seizures.

In a series of 104 temporal lobectomies, 85 cases fall within the excellent or good therapeutic evaluation groups.

In the laboratory, the anterior mesial thalamus was enucleated by microsurgical technique in adult Rhesus monkeys. Thereafter, the ipsilateral hemisphere exhibited high voltage sharp waves and spikes for as long as nine months. This epileptogenic activity could be exaggerated by the use of intravenous Metrazol, but even with the use of such convulsants was restricted to the side of thalamectomy. By callosal section and mesencephalic division of the ascending reticular system, the epileptogenic activity was restricted to the hemisphere in which thalamectomy was performed. These findings suggest that the anterior mesial thalamus has an inhibitory role and acts so as to raise convulsive threshold.

Involuntary Movements

In the laboratory, an attempt has been made to outline the upper border of the nucleus ventralis lateralis in the primate thalamus by use of a preparation in which the superior cerebellar peduncle was sectioned in two monkeys and one chimpanzee, thus resulting in degeneration which was stained by the Nauta-Gygax method. Reconstruction of the first specimen showed degeneration of fibers extending well forward, probably into the posterior portion of the ventralis anterior as well as the ventralis lateralis. In the anterior portion the degenerating fibers reached as high as the junction of the upper middle thirds of the thalamus height. In addition, template reconstructions of a chimpanzee brain prepared with Marchi method after section of the superior cerebellar peduncle, showed degeneration only in the lower half of the thalamus. Fine material was present in the upper portion of the thalamus. A study of the topography of the potential field about the lateral gastrocnemius-soleus nucleus as fired by antidromic shocks was undertaken in order to further understanding of the magnitude of recordable field as related to implanted depth electrodes.

Twenty-five normal human brains have been imbedded in jeltrate after marking the commissures and preparing for section in the transverse plane

and horizontal plane with reference to the intercommissural line. Material of this type serves as a basis for anatomical localization of lines in stereotaxic treatment of involuntary movements.

In the clinic, the fronto-striate arrest response has been studied in 30 individuals. This response was characterized by inability to carry out movement, including speech. It has been obtained in the left hemisphere of 18 persons and the right hemisphere in 12. The response is a relatively high threshold, having a mean stimulus level of 8 ma. Ancillary phenomena consisting of mood change, impaired auditory recall during stimulation, post-stimulation confusion and spontaneous inappropriate speech, and contraversive movement of the eyes and head have been obtained. It seems clear that seizure activity is not a feature of this arrest. EEG studies have also been made of the effect of lesions in the basal ganglia and thalamus and a comparison of depth and scalp activity during normal and induced sleep has been possible through use of this patient material. Patients with thalamic lesions are subject to intensive psychological testing immediately after coagulation and one year hence.

Cerebral Edema

By means of a standardized method of intracarotid injection of injurious solutes, blood-brain barrier damage, characterized by abnormal extravasation of acid dyes, was produced within one hemisphere of the rabbit, while the other served as a control. Comparative assay of the chloride and water content of the same brain was performed varying the type and concentration of the injuring solute and the time interval between injury and termination of the experiment. Findings from this study indicate that the effect of slight or moderate mercurial blood-brain barrier damage is expressed in significantly decreased chloride content in the hemisphere showing extravascular passage of tracer dyes. This suggests a functional disturbance in exchange mechanisms operating at the blood-brain interphase and not a mere leakage through ruptured blood-brain barrier structures. On the other hand, stronger mercurial injury resulted in the development of edema which was associated with an increase in both chloride and water content.

Unilateral blood-brain barrier injury was produced by an intracarotid injection of mercuric chloride, penicillin, or sodium acetrizoate. This injury was followed by a systemic administration of combinations of two different fluorescent and radioactive tracers: (1) red-fluorescent Rhodamine B labeled albumin with green-fluorescent fluorescein labeled albumin; (2) red fluorescent Evans Blue-albumin complex with FLS; (3) EBA with green fluorescent fluorescein labeled gamma globulin FLGG; (4) RLA with FLGG; (5) sodium fluorescein or FLA with C^{14} sucrose; (6) sodium fluorescein or FLA with C^{14} inulin; (7) sodium fluorescein with C^{14} methyl-o-glucose. Combinations of red and green fluorescent albumins showed no separation in the distribution pattern on the damaged side, but simultaneous administration of red fluorescent albumins and green fluorescent globulins revealed in numerous instances a distinct separation in distribution of protein tracers on the damaged side. In slightly or moderately damaged areas, numerous blood vessels were surrounded by red fluorescence. The perivascular exudates

and globules showed a range of color depending on relative concentration of respective tracers. C^{14} inulin appeared to penetrate in damaged areas more extensively than sodium fluorescein or FLA. An interesting result was obtained in sodium fluorescein-C^{14} methyl-o-glucose combination. Whereas in moderate or severe blood-brain barrier damage, both sodium fluorescein and C^{14} methyl-o-glucose spread intensely from the injured vessels, in very slight blood-brain barrier damage in which no abnormal passage of sodium fluorescein could be detected, there was a distinct inhibition of the normal transport of methyl-o-glucose from the blood into the brain tissue. Most of this experimental work was done in the rabbit, but additional and different studies were carried out in the shark in which lemon and nurse sharks provided by the Lerner Marine Laboratory, were subjected to cerebral and cerebellar lesions produced by cold, heat and chemical substances. All of these animals exhibited a striking resistance of brain tissue to injury. Tissue surrounding the lesion showed no, or very little, evidence of either edema or extravasation of the Evans Blue tracer. When a dose of mercuric chloride far exceeding that used in rabbits for disruption of the blood-brain barrier was used in sharks, there was no evidence of extensive parenchymatous extravasation of the tracer. The alteration of the blood-brain barrier was demonstrable only in the intense staining of the vascular walls with the Evans Blue tracer. Sharks subjected to intraventricular infusion of Evans Blue revealed passage of this tracer into the whole ventricular system, but there was no passage of the tracer into the subarachnoid spaces surrounding the brain and cerebellum.

In another study, the effect of systolic blood pressure on the dynamics of brain edema was investigated by alteration of the blood pressure in cats by means of hyper- and hypotensive drugs administered prior to production of brain edema. Edema was produced by application of a cooled metal plate to the exposed cerebral cortex. It was shown that the lowering of the blood pressure can almost completely prevent the development of edema.

Brain Tumor

Six patients with glioma and two with meningeal leukemia have received four perfusions each with methotrexate. While the drug is very effective in meningeal leukemia, it is not the drug of choice for intraventricular application.

Language and Memory

In this category, the phonemic aspects of induced dysphasia and normal naming errors have been investigated. The present technique is based on a study in which it was found that the type of initial and final phonemes in the nouns influenced the number of dysnomic errors made by epileptic patients during the carotid Amytal test. Thirty subjects were tested twice in the naming of 72 pictures of objects projected rapidly on a small screen. The greater the number of sounds, or phonemes, in the word, the greater were the number of errors in the object-naming task. The number of syllables, however, was not significantly related to the errors. Word frequency for the correct nouns was also a relevant factor in that objects with more common names

produced fewer errors than objects with rarer names. However, the substituted nouns were not of higher frequency than the correct nouns. In addition, when 24 subjects were asked to rate objects for familiarity, their ratings were found to be related to word frequency in the language but not to the errors obtained in the object-naming task. None of the aspects of the errors found with the speeded task with normal people were relevant to the errors observed originally during the carotid Amytal tests on epileptic patients. It seems that Amytal may disrupt some auxiliary component of a system in the hemisphere for speech, while speed may overload some other component.

A language training system has been developed in which 2,500 words of useful language can be transmitted to a previously naive subject in approximately 75 teaching hours. The catecholamine, eosinophil and various physiological responses of these subjects have been recorded and programed. It is intended that this system be used with patients who have undergone right or left therapeutic temporal lobectomy in an attempt to correlate language learning, lobectomy and the side of lobectomy.

Based on animal work completed last year, a test designed for memory mechanisms in man has been completed in this period. This apparatus separates the two factors of delay and complexity and thus permits their independent analysis. It will be used on patients with right or left temporal lobectomies.

Effects of Low Temperatures

It has been shown that freezing cold can produce completely reversible occlusion of blood flow in vessels larger than 2 mm. diameter without significant damage or risk of thrombosis for periods up to 30 minutes. This is true for both arteries and veins.

In another study a relatively selective method for brain cooling was used in conjunction with a system for controlled hypotension in which the animal's circulating blood volume was reduced by 'reserving' the blood within a reservoir of extracorporeal circulation for a predetermined time period. When the need for hypotension was over, the blood was returned to the vital circulation. In this combined methodology, cranial surgery at low temperatures and under a relatively bloodless condition, with recovery of the experimental animals, has been obtained.

Neurosurgical Monitoring

Nineteen normal subjects have been subject to comprehensive recording and the acquired data processed. Sixteen of 24 possible correlations between palmar skin temperature and EEG frequency, as recorded from occipital leads, are significant at the 5 per cent level. Correlation between left palmar lateralization and right EEG findings was significant in four out of six subjects at the one per cent level. Fifty-one patients coming to operation have been monitored for clinical purposes so that the operative course could be recorded in equipment and observed in progress.

Thirteen patients with 14 thalamic electrodes have been studied, as have six patients with two bilateral cingulum electrodes and nine medial temporal electrodes. Amygdala and uncal electrode stimulation was usually followed by respiratory depression and bradycardia.

A moderate increase in plasma 17-OH-CS levels from 11 to 18 mg.% was observed following amygdaloid stimulation, which is similar to changes observed in the Rhesus monkey. No delayed or prolonged suppression of 17-OH-CS levels as seen in the monkey was observed, however, following hippocampal stimulation in an epileptic patient. It is interesting that normal basal 17-OH-CS levels are observed in these patients, indicating that they are in a relatively unstressed condition at the time of the test.

Microbial Analysis of Neurosurgical Environment

The microbial environment in two architecturally distinct neurosurgical theater situations has been studied, using seven cases in one situation and 20 cases in another as the basis for observation. In the uninhabited environment, counts of one or less organism per four cubic feet of air per two-hour sample were obtained. In an 'active' environment, air counts ranged from a high of 11 to less than one bacteria per cubic foot for a 15-minute sampling period. Of 508 surface cultures and 38 air samples taken during seven neurosurgical cases, no hemolytic coagulase positive Staphylococcus aureus was identified. In further studies of 20 additional cases in the new neurosurgical suite, a Staphylococcus which was DNase positive and hemolytic was identified and eliminated. In addition, these studies were helpful in analysis of the plenum ventilation system, indicating a need for correction because of a defect in current flow.

Summary

The future of the categorical projects is presented in detail at the conclusion of each report. In summation, this Branch has principal tasks in the operating room investigation of patients by means of electrophysiological techniques. The more sophisticated use of the physiological monitoring system, in conjunction with the data programing, is intended to provide the surgeon with a capability for more precise clinical predictability of individual operating course, as well as correlations of physiological information, some of which are now being made. The human physiological profile thus obtained will be correlated with programed observations made on the ward unit and the biochemical profile which the Department of Clinical Pathology now obtains. It is hoped that this combined data recording and acquisition system will then be applied to the categorical disease entities under study, such as epilepsy, involuntary movements, head injury, brain tumor, etc. The program in head injury should extend its biochemical support and develop a more sophisticated physical basis. This project, and those in epilepsy and involuntary movements, are, to a large extent, concerned with brain stem mechanisms, and thus a kind of functional coherence is evident as a consistent and common characteristic. It is hoped that as these evolve, significant contributions to knowledge of the human reticular system can be made. The head injured patient is essentially a problem in coma, the epileptic patient a problem in alteration

in consciousness, and the involuntary movement patient a problem in relevant, although not identical, thalamic mechanisms. Perhaps through anatomical, physiological, and surgical methodology, some further understanding of brain stem mechanisms in general, and reticulo-thalamic mechanisms in particular, will be forthcoming. Problems in communication and the use of information theory are now at hand, and an increasing use of sophisticated linguistic techniques, as well as data processing methods, will be attempted. The conventional, and ever present neurosurgical problems of cerebral edema, hemostasis, and infection remain as challenging targets. Extracorporeal circulation techniques, hypothermia, study of blood clotting, and specific technical sophistication of design for the reduction of infection will continue as high priority goals. It is hoped that in addition to the newly developed surgical techniques of brain splitting, thalamectomy, etc., the microsurgical methods can be extended to cerebral blood vessels so as to approach problems of the intracranial carotid circulation.

Serial No. NDB(I)-54 SN/OC 100(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Epileptogenic Mechanisms in the Brain of Man and Other Primates

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: J. Van Buren, M.D., A. Ommaya, F.R.C.S., I. Klatzo, M.D., R. Farrier, M.D., C. Ajmone Marsan, M.D., T. Milhorat, M.D., D. Sadowsky, F. MacDonald, R.N.

Cooperating Units: Clinical Pathology Department, CC; Biometrics Branch, NINDB

Man Years: (computed for the 12-month period)
Total: 2.6
Professional: 1.6
Other: 1

Project Description:

Objectives:

a. To study causal mechanisms of epileptic seizures in man and other primates.
b. To study the electrographic characteristics of epileptogenic activity in the brain of man and other primates.
c. To study the approved methods of surgical therapy for these lesions and develop new therapeutic methods.

Methods Employed:

a. Clinical neurological examination.
b. Special radiographic and other contrast examinations.
c. Electrographic, including electrocorticographic, examination.
d. Electrophysiological techniques as indicated.
e. Experimental epileptogenic lesions.
f. Hypothermia.
g. Cerebral drug deposition and scintillation counting of tagged drugs.
h. Histological and chemical examinations as required.

Major Findings: During the period of this report, 93 patients with cerebral seizures were studied in the operating theaters and on the ward. (Report period 1 July 1964 - 22 April 1965.)

One hundred and four cases subjected to therapeutic temporal lobectomy for seizures have been studied. The data from these patients has been programed in accordance with a coding system described in last year's report. There were 20 data points per person. In consideration of some of the data points, it is interesting to note that a severe head injury is significant when the severity indicated a blow sufficient to cause loss of consciousness. This occurrence was particularly related to inability to make a good social adjustment after hospitalization. Similarly, interictal disturbances, especially of social behavior, the presence of psychotic manifestations, the presence or persistence of neurological deficits of either sensory or motor systems, and combinations of the foregoing are highly significant in the results in social adjustment. This is interpreted to indicate that the presence of such interictal disturbance does not militate against a good result in terms of number and severity of seizures and the EEG manifestations, but that the social adjustment of such patients is ordinarily poor and that this result is often the crucial factor in deciding the benefit of surgery in such cases of temporal lobe epilepsy. In another data point sub-group, that of no tumor versus tumor, it is quickly apparent that the presence or absence of tumors discovered incidentally, the variety of seizures, the pattern of seizures at the time of surgery, and the consistency of the seizure pattern in the history of the disease are not significant in terms of the results of surgery. The non-significance of tumors in this series was surprising, but this result may be spurious in view of the very small number of cases in which tumors were found. One explanation of this finding could be that such cases do not form an etiologically significant group in patients with temporal lobe epilepsy. The results of surgery with relationship to continuation of seizures indicate that 85 out of 104 patients fell within the excellent or good groups.

In selected patients with severe unlocalized (centrencephalic) cerebral seizures, a clinical trial of stereotaxically placed depth lesions in the Fields of Forel and the zona incerta is being pursued. Stereotaxically placed lesions in the amygdala are also being made in certain cases of cerebral seizures poorly localized to one hemisphere. Preliminary results are encouraging but too few to allow definitive conclusions.

In the laboratory, the anterior mesial thalamus was enucleated unilaterally in a series of adult Rhesus monkeys and the animals were studied for a postoperative period of nine months. In a number of these subjects, a split-brain lesion consisting of division of the corpus callosum and midline commissures was combined with the unilateral thalamic lesion. All lesions were made directly by microdissection technique described in a previous report. In addition, in a second series of animals, the cerebral hemisphere on the side of thalamectomy was subjected to hemisection of the mid-brain tegmentum, alone or in combination with the split-brain lesion.

The EEG records from animals with unilateral thalamic lesions were characterized by dysrhythmia which was most pronounced over the thalamectomized hemisphere. High voltage and sharp waves were recorded over the operated hemisphere and some bilateral symmetry was present. When intravenous Metrazol was given slowly, clonic movements of the opposite side of the body developed before a generalized attack. In animals where unilateral thalamectomy was combined with split-brain lesion, hypersynchronous EEG patterns were recorded from the isolated hemisphere and spontaneous myoclonus was observed in the opposite extremities. When Metrazol was administered in doses as low as one-tenth the usual convulsive dose, seizures occurred, as indicated by spikes from the separated hemisphere and clonic movements confined to the opposite side of the body. Between attacks, these animals appeared fully alert and normal in spite of the EEG records.

The drug deposition studies have been continued, utilizing curare, and the studies of dilantin levels in brain around a penicillin lesion have been reconfirmed.

Significance to Bio-medical Research and Program of the Institute: These observations have contributed to the knowledge of epilepsy and its treatment.

Proposed Course of the Project: The categorical nature of the patient material will be modified so that the primary selection criteria are etiological. Thus, post-traumatic epilepsy will be selected as a principal etiological category. In the past, selection criteria have been derived from functional anatomy related to the origin of the attacks. It is proposed that the post-traumatic epilepsies pass through the same study mechanisms as have been used in the past, and in addition, some highly selected cases will be subjected to Echlin techniques in the operating theater where the clinical situation warrants. It is anticipated that the effects of anticonvulsants at low temperatures will be observed in the future, and the micro- and depth electrode studies will be continued. Similarly, in the laboratory the effects of large cerebral tract severance will be observed and clinical operative procedures devised from this experimental surgery.

Part B included: Yes

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Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Ommaya, A. K. and Sadowsky, D.: A system of coding medical data for punched card machine retrieval. I. As applied to epilepsy. Epilepsia 5: 192-200, June 1964.

Serial No. NDB(I)-54 SN/OC 101(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Functional Representation in the Temporal Lobe of Man and Higher Primates

Principal Investigators: Maitland Baldwin, M.D. and A.K. Ommaya, F.R.C.S.

Other Investigators: J. Van Buren, M.D., R. Farrier, M.D., H. Lansdell, Ph.D., G. Ojemann, M.D. F. MacDonald, R.N., R. Wadeson, M.D. J. W. Mason, M.D.

Cooperating Units: Section on Animal Behavior, NIMH; Clinical Pathology Dept., CC; Biometrics Branch, NINDB; Dept. of Neuroendocrinology, WRAIR, WRAMC; Berlitz School of Languages.

Man Years: (computed for the 12-month period)
Total: 2.3
Professional: 1.3
Other: 1

Project Description:

Objectives: To further understanding of functional representations within the temporal lobe and related structures of man and higher primates.

Methods Employed:

a. Electrical stimulation and recording from human, chimpanzee, and monkey temporal lobes; directly, after operative exposure; indirectly, by depth electrodes and scalp recordings.
b. Ablation of all or parts of the temporal lobes and limbic systems.
c. Surgical interruption of pathways connecting these systems.
d. Use of drugs for stimulation and suppression, or other effects, on these systems.
e. Test situation, utilizing machine or automatic programing.
f. Other psychological and physiological examinations.

Major Findings: Based on the animal work reported last year, the new development of a test design for memory mechanisms in man has been completed, and the apparatus will be in operation shortly. The tests designed allow the separation of the two factors of "delay" and "complexity" and their independent analysis. This ability to separate delay and

complexity of a memorandum has not been successfully achieved hitherto.

Initially, the apparatus will be tried on normal individuals and subsequently on patients with right and left temporal lobectomies, controlling for such factors as age, sex, and presence or absence of EEG abnormalities in the intact temporal lobe.

Patients with temporal lobe disease requiring diagnostic lateralization of epileptogenic processes were subjected to depth electrode implantation with a view to recording from the mesial temporal structures so as to lateralize significant, predominant epileptic discharges. Following surgery, these patients were ambulatory and available for physiological monitoring. Coincidental with recording from the depth electrodes, scalp EEG, skin temperature, rectal temperature, blood pressure, EKG, and respiratory rate were monitored. These data were subject to digital conversion and programing in accordance with a previously designed system. In addition, time-related catecholamine studies were undertaken, as were evaluations of recent memory (by visual display test). Two of these cases are worthy of special mention. In one, the depth electrodes were localized on the left in the uncus; on the right, in the pes hippocampus. In the other, they were located on the left in the mesial amygdala; on the right, in pes hippocampus. Stimulation with 1 ma. or less was followed by bradycardia and respiratory arrest in uncus and mesial amygdala, but not in pes. Stimulation of pes and uncus with similar current values produced significant alteration in aptitude on recent memory testing, but this was not found in stimulation of mesial amygdala.

A moderate increase in plasma 17-OH-CS levels from 11 to 18 ug.% was observed following amygdaloid stimulation, which is similar to changes observed in the rhesus monkey. No delayed and prolonged suppression of 17-OH-CS levels, as seen in the monkey, was observed, however, following hippocampal stimulation in an epileptic patient. It is encouraging that normal basal 17-OH-CS levels are observed in these patients, indicating that they are in a relatively unstressed condition at the time of the test.

Two patients undergoing temporal lobectomy have now been extensively studied through a one-year followup period. The lowering of urinary 17-OH-CS levels noted during the first postoperative month was found to be transitory, since levels returned to the preoperative range at the time of the one-year followup study. At this time no significant changes were observed in urinary epinephrine, norepinephrine, or aldosterone levels. The most marked changes observed were in the urinary estrogen levels, which appear to be substantially elevated in both patients one year postoperatively.

A new study of language performance in temporal lobectomized patients has begun. Patients who have had right or left subtotal lobectomy are being readmitted for total assessment, and from among those with normal full-scale performance on Wechsler-Bellevue test, a sub-group is being selected at random from rights and lefts for training in a language foreign to them. A system has been devised in which a person of normal intelligence and useful hearing and vision can acquire approximately 2,500 words of a

language for conversational purposes within 75 hours of instruction. This system has been developed through the cooperation of a series of normal individuals who have undergone various trial teaching periods. In particular, three groups of normals, in the numbers of ten, five and sixteen, have undergone intensive training in Spanish or Vietnamese. Other languages taught for the purposes of this system include French, German, Russian and Swahili. The normal groups undergoing language training have been subjected to physiological monitoring and catecholamine estimation, as well as eosinophil counts in order to assess the stress of this training. In general, the ketosteroid output increases moderately, while the eosinophil count falls perceptively in the first two days of instruction, after which normal values return. In one case, characterized by general ineptitude, the ketosteroid values and eosinophil values were quite abnormal throughout, as were the polygraph recordings. Foreign language aptitude tests, Minnesota Memory, McGill and NINDB tests have been given to these subjects and all have been graded pedagogically for foreign language aptitude before and after training. The average language acquisition has been 2,500 words in the 75 hours of teaching.

Significance to Bio-medical Research and Program of the Institute: This project provides opportunity for further study of functional representations of language, memory and the limbic counterparts of the temporal lobe systems. In its present form, this project provides an opportunity for assessment of language function as related to specific surgical lesions of the temporal lobe in man.

Proposed Course of the Project: The depth electrode and other electrophysiological studies of the temporal lobe will continue. Patients with extensive hippocampal lesions are still being sought for participation in the so-called memory parts of this project. It is hoped that the patients who require therapeutic temporal lobectomy on either left or right sides will participate in the language training system so that at operation they can be language tested in the traditional fashion in English, as well as in the acquired tongue. Such a testing will also include ECG, EMG, and phonemic analysis of the speech. This will be correlated with the study of language acquisition capabilities in right and left temporal lobectomy patients as noted above.

Part B included: No

Serial No. NDB(I)-59 SN/PN 601(c)
1. Surgical Neurology Branch
2. Primate Neurology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Effect of Radio-Frequency Energy on Primate Brain and other Biological Systems

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: S. A. Bach, M.D., W. Pearlman, B.S.C., F. MacDonald, R.N., I. Klatzo, M.D.

Cooperating Units: Army Medical Research Laboratory, Ft. Knox; Instrument Engineering & Development Branch, DRS, NIH; Institute for Research, State College, Pa.

Man Years: (computed for the 12-month period)
Total: 1.1
Professional: .9
Other: .2

Project Description:

Objectives: Same as NDB(I)-59 SN/PN 601(c)

Methods Employed: Same as NDB(I)-59 SN/PN 601(c)

Major Findings: Same as NDB(I)-59 SN/PN 601(c)

Proposed Course of the Project: This project is discontinued.

Part B included: No

Serial No. NDB(I)-60 SN/NA 702(c)
1. Surgical Neurology Branch
2. Section on Neuroanesthesiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Experimental Hypothermia

Principal Investigator: Maitland Baldwin, M.D.

Other Investigators: T. Ohta, M.D., J. Sagarminaga, M.D., A. Ommaya, F.R.C.S., R. Farrier, M.D., F. MacDonald, R.N.

Cooperating Units: Department of Anesthesia, CC; Instrument Engineering and Development Branch, DRS, NIH

Man Years: (computed for the 12-month period)
Total: 4
Professional: 2.6
Other: 1.4

Project Description:

Objectives:

a. To study the effects of low temperature on the brain.
b. To study various related physiological parameters.
c. The development of topical and selective cooling techniques potentially available for clinical application.

Methods Employed: The methods employed are body surface cooling, topical cooling of the exposed brain, and/or spinal cord, intravascular perfusion, and combinations of these.

Major Findings: In 133 healthy mongrel dogs, body weight 12-26 kg., a relatively selective cooling of the brain was performed in conjunction with controlled hypotension.

Each animal was anesthetized, being induced with pentothal (25 mg./kg.), intubated and maintained with halothane-oxygen and intermittent positive pressure respiration with the use of a Bird respirator. In addition, each animal received heparin (2 mg./kg.). A venous catheter (#18) was put into the inferior vena cava in the thoracic cavity through the left femoral vein. An arterial catheter (#14) was passed through the left femoral artery to the branching of the brachiocephalic and left subclavian arteries from the arch of the aorta. The position of the tip of the catheter was confirmed by thoracotomy before or at the end of the experiment.

Just proximal to the arterial catheter, a tube containing 15 ml. of 50 per cent Hypaque was attached parallel to the tube coming from the bubble trap and clamped off from the usual circuit. At any chosen time of the cooling phase, the side tube was opened so as to let the radiopaque medium flow into the artery at the velocity and force of pumping. A serial exposure apparatus (Sanchez-Perez) was used and 12 films were taken at the rate of two films per second. A majority of the radiopaque medium could be seen to ascend to the brain with 60 mmHg. of arterial pressure and flow rates of 22 and 10 ml./kg./min., respectively.

The shunt of the extracorporeal circulation was veno-arterial, and the average rate of flow was estimated at 25 ml./kg./min., although it was much more than the average at the beginning, and progressively reduced towards the end of the cooling phase. Drainage of venous blood into the oxygenator was by gravity. The inflow blood was cooled to 20° C. and maintained at this temperature throughout the cooling phase.

The arterial, systemic venous, and CSF pressures and lead II of the electrocardiogram were recorded on a Sanborn 150 multi-channel recorder. Statham transducers P23AA and P23BB were used. Temperatures in both cerebral hemispheres (1-1.5 cm. in depth in the frontal region), esophagus, rectum, inflow and outflow blood, and the blood in the superior vena cava were observed every five minutes, with the use of several Yellow-Springs thermistor probes and a mercury thermometer.

When the brain was cooled to 28° C., the arterial pressure was reduced to a level of approximately 30 mmHg. (ranging from 20 to 40 mmHg. F) by reducing the inflow of the cooled blood to an average rate of 6 to 10 ml./kg./min. and by allowing controlled bleeding from the venous catheter (about 100 to 300 ml.). The animal was maintained for 30 minutes in this condition, then gradually rewarmed to an esophageal temperature of 34° C., at which time the extracorporeal circuit was interrupted. The arterial catheter was withdrawn to the abdominal aorta just before rewarming in order not to warm the brain faster than the rest of the body.

These results confirm last year's finding that relatively selective brain cooling is possible by high aortic catheterization, and in addition demonstrates that such cooling applied in combination with controlled hypotension provides a relatively bloodless surgical field in the brain under conditions consistent with optimum recovery of the experimental animal.

Significance to Bio-medical Research and Program of the Institute: The methodology described may provide for a clinically reproducible method for brain cooling which will obviate the dangers of cardiac instability and heparinization, as well as provide for control of bleeding.

Proposed Course of Project: The effects of low temperature on the brain will be studied both for potential clinical applications and physiological interest. (1) A technique for selective cooling of one hemisphere is now being developed. In conjunction with the use of this technique and that described above, a study of the effects of low temperature and other unusual factors on the circle of Willis will be undertaken. (2) A study of apparent

relationship between hypothalamic control and artificially induced hemispheral temperature will be undertaken.

Part B included: No

Serial No. NDB(I)-63 SN/PN 1024(c)
1. Surgical Neurology Branch
2. Section on Primate Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Surgical Approach to the Third Ventricle

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: T. Milhorat, M.D., R. Farrier, M.D.
F. MacDonald, R.N.

Man Years: (computed for the 12-month period)
Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: Same as NDB(I)-63 SN/PN 1024(c) for period ending June 30, 1964.

Methods Employed: Same

Major Findings: Same

Proposed Course of the Project: This project is complete.

Part B included: No

Serial No. NDB(I)-65 SN/OC 1205(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Neurosurgical Monitoring

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: Robert Farrier, M.D., George Ojemann, M.D., Ayub Ommaya, F.R.C.S., John Van Buren, M.D., Gerald Cohen, B.E., Paul Fedio, Ph.D.

Cooperating Units: Instrument Engineering & Development Branch, DRS, NIH; Analysis & Programming Section, CDPB, DRS, NIH.

Man Years: (computed for the 12-month period)

Total:	2.7
Professional:	1.7
Other:	1

Project Description:

Objectives:

1. Physiological parameters of neurosurgical operations.
2. Processing of data thus derived.
3. Estimation of clinical and physiological significance of correlations obtained from such data.
4. Physiological effects of cortical and subcortical electrical stimulation.

Methods Employed:

1. Application of the Sanborn Physiologic Data Monitoring System.
2. Recording from patients during operation.
3. Recording from non-operated patients.
4. Recording from normal controls.
5. Data programming by the Computer Branch in accordance with a collaborative plan.

Major Findings: Seven normal controls have been monitored for a time period which varied from a minimum of four to a maximum of six hours. Six of these subjects yielded useful data. Sixteen of 24 possible correlations

between palmar skin temperature and EEG frequency (as recorded from occipital leads with a counter based on signal amplitude) are significant at the 5 per cent level. A correlation between left palmar lateralization and right EEG findings was significant in four out of six subjects at the 1 per cent level.

In addition, recordings have been obtained from 12 normal subjects with an average individual recording time of one hour each. These subjects have been subjected to a moderately stressful learning experience (language). The physiological data obtained from these is being correlated with psychological performance testing information, and ketosteroid and eosinophil values. The data is also being related to diurnal-nocturnal cycles. In the experience with these normal subjects, a simple test of object naming and post-distractional recall has been developed and used.

An attempt has been made to relate the latter test to effects of lateral amygdala stimulation at a threshold of 5 ma. Such stimulation was followed by prompt loss of post-distractional recall. It is interesting that this stimulus did not interfere with undistracted recall.

Thirteen patients with 14 thalamic electrodes have been studied, as have six patients with two bilateral cingulum electrodes and nine medial temporal electrodes.

Low threshold stimulation of anterior thalamus, adjacent internal capsule and nearby caudate head produced frequent inhibition of involuntary respiration. Amygdala and uncal electrode stimulation was usually followed by respiration depression and bradycardia. The cingulum responses were variable from patient to patient, but consistent in each patient. These, and in particular the results of the thalamic electrode stimulations, are presented elsewhere in this annual report in some detail.

Fifty-one patients coming to operation have been monitored for clinical purposes so that the operative course was recorded in the equipment.

Significance to Bio-medical Research and Program of the Institute: This project has scientific, as well as clinical significance. It is designed to provide physiological recording of various functions following electrical stimulation of the human nervous system, and under stimulation through various dependent variables. It now provides the neurosurgeon with a prototype system for a sophisticated recording of a patient's operative course. Through data processing, it is hoped thatit may provide a measure of predictability for the surgeon of the patient's future course in the operating room, as well as significant physiological correlations.

Proposed Courseof the Project: It is proposed that further baseline data be obtained from normal subjects and that the patient material evaluated by the monitoring system will be diversified and enlarged. A plan

has been formulated for correlation of data obtained from these systems with biochemical profile data obtained in clinical pathology. It is hoped that this combination and correlation will provide further significant relationships.

Part B included: No

Serial No. NDB(I)-65 SN/OC 1206(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Microbial Characteristics in a Neurosurgical Environment

Principal Investigator: Maitland Baldwin, M.D.

Other Investigators: R. J. Weatherby, F. MacDonald, R.N.

Cooperating Units: Environmental Services Branch, DRS; Employee Health Service Branch, CC.

Man Years: (computed for the 12-month period)
Total: 1
Professional: .7
Other: .3

Project Description:

Objectives: Analysis of salient microbial features in a neurosurgical operating room environment.

Methods Employed:

a. Personnel and environmental microbial sampling.
b. Analysis of physical environment.
c. Analysis of personnel structure.

Major Findings: The microbial environment in two architecturally separate neurosurgical theater situations has been studied. The 'passive' characteristics of each environment were evaluated with particular regard to air sampling for microbial content, air flow pattern, temperature, humidity, convection surfaces, heat sources, etc. The 'active' characteristics of the environment were then sampled by means of personnel cultures, floor cultures, cultures from scrub sink faucets, etc., and various other elements of the surgical area, such as irrigation solution, and progressively from the incision site by a suction sample technique, as well as a progressive air sampling system. The 'passive' characteristics demonstrate that the environment, as such, contains one or less organism per four cubic feet of air per two-hour sample. Air samples of the 'active' characteristics show organism counts ranging from a high of 11 to less than one bacteria per cubic foot for a 15-minute sampling period. Of the 508 surface cultures and 38 air samples taken during the seven neurosurgical cases, no hemolytic coagulase positive Staphylococcus aureus was identified. The skin of patients sampled yielded Staphylococcus albus, and it is interesting that cultures taken from

the floor during operation, and thus as part of the 'active' characteristics, did not show any significant change in the number of micro-organisms as compared with samples taken as part of the 'passive' characteristics. The bacterial level is, therefore, remarkably low.

However, in the continuation of these studies through a series of 20 additional cases in the new neurosurgical suite (or second architectural area), a Staphylococcus which is DNase positive and hemolytic has been identified. In addition, studies of the 'passive' characteristics of the new environment have revealed a rare, but important, discrepancy in the plenum ventilation system which periodically has reduced its flow and pressure characteristics below those sufficient for maintenance of a centrifugal decline of pressure and flow away from the operating table. In further analysis of these problems, a comprehensive analysis of physical characteristics of the environment have been studied with methods cited above, and also all personnel have been isolated in a specially designed tent which provides for air sampling of skin organisms resulting from skin shedding. Personnel and patients have been sampled in selected skin areas. Samples have been taken from the ward environment, as well as operating room environment. The Staphylococci, DNase positive, source has been identified and will be eliminated.

Such studies provide a patient safeguard and are, therefore, a patient service function. However, they constitute a research project as well, since research and development of and for the neurosurgical operating room environment is an essential function of this Branch. Through these studies, progressive redevelopment of the facility, and perhaps also support in solution of similar problems arising in other facilities, becomes feasible.

Significance to Bio-medical Research and Program of the Institute: This methodology provides for continued sophistication in the development of modern neurosurgical operating room environments.

Proposed Course of the Project: The project will be continued, both as a patient safeguard and for the development of a more sophisticated operating room system. It is proposed that sampling of personnel and material will continue on a schedule, and new and more effective sampling devices will be developed. Relationship between flora of the environment and surgical wound characteristics will be made. The efficacy of engineering and surgical techniques will be evaluated against this microbial methodology.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Baldwin, M., Weatherby, R.J., and MacDonald, F.D.S.: Microbial characteristics in a neurosurgical environment. Hospitals, J.A.H.A. 39: 71-78, March 1965.

Serial No. NDB(I)-62 SN/OC 906(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Effects of Cold and the Relation of Temperature to Functions of the Central Nervous System

Principal Investigator: A. K. Ommaya, F.R.C.S.

Other Investigators: John Coe, M.D., E. Flamm, M.D., S.David Rockoff,MD and Maitland Baldwin, M.D.

Cooperating Units: Diagnostic Radiology Dept. C.C.

Man Years (computed for the 12 month period)
Total: 2.2
Professional: 1.5
Other: .7

Project Description:

Objective: This remains as for calendar year 1963 with the following additions:

a) A study of comparative temperature gradients in nervous and other tissues under various normal and abnormal conditions and in response to heating and cooling of small areas. (In cats)

b) A study of the effect of freezing temperature on blood vessels of varying calibre in animals. (Cats and monkeys)

c) A study of the effect of moderate hypothermia (28-32^{o}C) on the viscosity of blood in man and animal.

Methods Employed: In addition to those detailed in the report of 1963, the following instrumentation and techniques are being used or developed:

a) a 24-point multiple-pattern Thermocouple array for temperature gradient recording. This array consists of micro-Thermocouples arranged around a central probe shaft which can be used for heating, cooling or biopsy. The data is to be recorded through a Dymec Data acquisition system on punched tape, thus facilitating analysis.

b) a differential-temperature Thermocouple to study related problems in situations where the 24-probe array cannot be used.

c) angiographic X-ray demonstration of zones of freezing block and other changes in blood vessels of cats.

d) construction of a new system for focal heating and cooling within the nervous system.

Major Findings: The study on viscosity of blood after hypothermia has been discontinued due to lack of sufficiently consistent data with existing methods.

The study on blood vessels has been completed and is to be published in The Acta Neurochirurgica shortly. In summary it has been shown that freezing cold can produce completely reversible occlusion of blood flow in vessels larger than 2 mm diameter without significant damage or risk of thrombosis for periods of freezing occlusion up to 30 minutes. This is true for both arteries and veins. Further studies on vessels of smaller caliber are now being pursued.

The study of thermal gradient has been delayed by instrumental difficulties but is now re-established. However the results are not sufficient to warrant any conclusions at this stage.

A study of the effect of urea on the type of cerebral edema produced by freezing cold is also being pursued. This series of experiments is still at an early stage.

Proposed Course of Project: Same as NDB(I)-SN/OC 906(c).

Part B included: Yes[X] No[ ]

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Coe, J.E., and Ommaya, A.K.: The effect of extreme cold on major extracranial vessels (in press) Acta Neurochirurgica.

Coe, J.E., and Ommaya, A.K.: Evaluation of focal lesions of the Central nervous system produced by extreme cold. J. Neurosurg. 21: 6. 433-444, 1964.

Chase, R.A., Cullen, J.K., Jr., and Ommaya, A.K.: Modification of intention tremor in man (in press). Nature.

Serial No. NDB(I)-62 SN/OC 907(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Trauma to the Nervous System

Principal Investigator: A. K. Ommaya, F.R.C.S.

Other Investigators: E. S. Flamm, M.D., A. Hirsch, D. Goldman, M.D., R. Mahoney, D. Rockoff, M.D., and R. Wadeson, M.D.

Cooperating Units: Personnel Protection Section, D.T.M.B., Dept. of the Navy
Department of Radiology, C.C.
Laboratory of Biophysics, N.M.R.I.

Man Years (computed for the 12 month period)
Total: 3.1
Professional: 2.0
Other: 1.1

Project Description:

Objective: Same as NDB(I)-62 SN/OC 907(c).

Methods Employed: As for 1963 with the following additions. The proposed collaborative study of head injury patients could not be initiated due to lack of funds. It is hoped that this study will be carried forward in the coming year.

Major Findings:

A. Experimental Study: Statistically significant correlations between the production of cerebral concussion and the impulse of the blow have been made. (Impulse is defined as the area under the force curve directly recorded from the impact and the unit is lb. sec.) This experimental model of cerebral concussion in the monkey is now being tested under a variety of conditions in order to fully understand the mechanics of concussion. In addition to recording the impact force and velocity of blow, the linear acceleration of the head, intracranial pressure changes at various sites, and the displacement of the head in 2 axes is being carefully measured. Based on this data a theoretical model for experimental head injury is being developed. Due to the paucity of quantitative data on the rheological properties of the cranial contents, our initial effort envizages a discrete rather than a continuum model for accurate

prediction of the mechanical response. The first model is relatively coarse being a proposition that the brain stem and dura serve as springs, the cerebrum, cerebellum and the skull as masses, and the extra-cerebral liquids as dashpots. The total number of degrees of freedom is being kept at two. In the preliminary analysis of this model the constraints imposed by the craniospinal junction and the separation of finite rigid-body motions from deformations are being rigorously incorporated. Although this system is initially being treated as linear, it is recognized that the onset of non-linearity is unknown a priori and that concussion may be inherently non-linear, e.g., if it corresponds to some "temporarily irreversible" mechanical deformation at the membrane level.

It is hoped that funds will be available to award a contract to a mathematical consultant to develop this mechanical model applicable to primates. This will enable us to specify the properties for which it requires measured numerical values and to suggest experiments whereby the predictions from the theory may be verified.

In order to provide quantitative data on the behavior of nervous tissues under mechanical stress, a series of experiments on the frog sciatic nerve have been started. The frog nerve trunk is placed in a special chamber which allows the controlled application of either stretch or crush in a measured area of the nerve. Since these forces are directly measured, it is feasible to record the compound action potential of the nerve and to study its electrical behavior during tensile, compressive or shear strains of known amounts. These experiments are still in the pilot stage and much work remains to be done to determine the type of force to which the electrical properties of the frog sciatic nerve is most susceptible.

The radiological studies using carotid angiography have been extended to cover the immediate post-traumatic interval and the later effects of the blow. To date it would appear that the marked slowing of circulation described in last years' report (within the period of 15-30 min. after trauma) is of early onset (within 1-2 minutes of the blow) and appears to parallel the development and persistence of post-traumatic cerebral edema. However further work remains to be done to clarify this relationship and to understand the time-course of this effect of trauma on the cerebral circulation. Current experiments on this aspect include direct measurements of carotid artery pressure and flow.

The biochemical aspects of this study have not been pursued further due to the lack of technical support.

B. Clinical Research Program: Continuing observations on a few selected cases of severe head injury have given further support to a hypothesis postulated in 1963 that the adult patient after a severe head injury, without additional complications, displays an accelerated re-capitulation of his post-natal ontogeny. Treatment should therefore be aimed at ensuring that the transitions towards the "adult normal" stage be made as smoothly as possible. However this remains a hypothesis and until the proposed collaborative study

of head injured patients becomes a reality, it would appear impossible to study further the patterns of re-integration in the traumatized central nervous system with existing facilities.

Significance to Neurological Research: Same as NDB(I)-62 SN/OC 907(a).

Proposed Course of Project: As for 1963 with the following addition. The design of an ultra-high speed cineradiographic system utilizing field emission x-ray sources has been completed and will be shortly used to directly record and measure the deformations and displacements of intracranial structures at and immediately after impact trauma. These structures will be visualized either directly e.g., by "hypaque" angiography and "pantopaque" ventriculography, or indirectly by stereotaxic placement of iso-density radio-opaque particle in various brain and cord structures.

Part B Included: Yes/X/ No/ /

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B

Publications other than abstracts from this project:

Ommaya, A.K., Rockoff, S.D. and Baldwin, M.: Experimental concussion. A first report. J. Neurosurg. 21: 249-267, 1964. (April)

Rockoff, S.D. and Ommaya, A.K.: Experimental Head Injury. Cerebral angiographic changes in the early post-traumatic period. Am. J. Roentgenol. 91: 1026-1035, 1964. (May)

Honors and Awards

Elected Hunterian Professor of the Royal College of Surgeons of England. 1965. To deliver Lecture entitled : "Trauma to the nervous system".

Serial No. NDB(1)-63 SN/OC 1025(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Gliomas of the Nervous System

Principal Investigator: A.K. Ommaya, F.R.C.S.

Other Investigators: David Rall, M.D., M. Baldwin, M.D., R. Rubin, M.D., E. Henderson, M.D., I. Klatzo, M.D., F.G. Gieseke, and M. Bagan, M.D.

Cooperating Units: Section of Pharmacology, N.C.I.

Man Years (computed for the 12 month period)
Total: .8
Professional: .4
Other: .4

Project Description:

Objective: Same as for 1963

Methods Employed: Same as for 1963

Major Findings: To date six patients with glioma and two with meningeal leukemia have received approximately 4 perfusions each with methotrexate. The results would indicate that although very effective for meningeal leukemia, methotrexate is not the drug of choice for this technique. Animal tests of other cytotoxic agents are now in progress and these will be applied to further patients. This project is presently limited by the lack of adequate support in the laboratory for testing of drugs in experimental animal gliomas and in the clinic by the lack of sufficient beds. Currently only two beds are available for these patients.

Proposed Course of Project: As for 1963 with the following addition. From July 1965 a full-time Research Associate is being assigned to this project, working in the Pharmacology Laboratory of the N.C.I. but under the direction of the principal investigator and Dr. David Rall. This will enable the experimental support of this project to be enhanced.

Part B included: Yes/X/ No/ /

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Ommaya, A.K., Rubin, R.C., Henderson, E.S., Rall, D.P., Gieseke, F.G., and Bagan, M.: A new approach to the treatment of inoperable brain tumors (in press) D.C. Med. Annals.

Coe, J.E., Ommaya, A.K. and Dirlik, P.: Stereotaxic isotope scanning. (in press) J. Neurosurg.

Coe, J.E., Ommaya, A.K. and Dirlik, P.: An instrument for brain biopsy. (in press) J. Neurosurg.

Krueger, T.P., McFarland, J., and Ommaya, A.K.: Pyocele of the sphenoid sinus. (in press) J. Neurosurg.

Kastin, A.J., Lipsett, M.B., Ommaya, A.K. and Moser, J.M., Jr.: Asymptomatic hypernatremia. Am.J. Med. 38: 306-315, 1965.

Serial No. NDB(I) 56 SN/OC 303(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Study of the Functional Anatomy and Pathology of the Human Visual System

Principal Investigator: J. M. Van Buren, M. D.

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

(1) Due to the death of Dr. Theodor Wanko, the proposed electron microscope studies of the fiber counts in the human optic nerve and studies with papilledema have been abandoned.

(2) The material collected (14 pairs of retinae in serial section) for evaluation of the effects of papilledema upon the ganglion cell population, remains on file. It is awaiting evaluation.

Part B included: Yes No

Serial No: NDB(I)-56 SN/OC 304(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Effect of Lesions Upon the Function and Structure of the Human Central Nervous System

Principal Investigator: Dr. J. M. Van Buren

Other Investigators: P. I. Yakovlev, M. D.

Cooperating Units: Warren Anatomical Museum, Harvard School of Medicine

Man Years (computed for the 12 month period)
Total: 1.4
Professional: .5
Other: .9

Project Description:

Objective: The program makes use of the intrusion of disease upon the central nervous system as an "experimental lesion." At the present time, major emphasis is directed toward studies of the human thalamus with view to producing better anatomical control for therapeutic procedures upon the thalamus and basal ganglion.

Methods Employed: The following steps are contemplated:

(1) Define the readily identifiable nuclear groups of the thalamus in all three planes using photographs of Nissl and Myelin sections.

(2) Using template reconstructions, show the variations in the thalamic nuclear topography in each of the three major planes.

(3) Relate the variations in the nuclear outlines to the true brain measurements and the anterior and posterior commissures.

(4) In some thrity full brain reconstructions, from Nissl and Myelin sections, demonstrate the relationship between focal cortical lesions and thalamic degeneration. These would be illustrated as full template series showing in detail the outline of the lesion as well as the thalamic degeneration.

(5) Retrace the ontogenetic development of the adult thalamic nuclear masses. This again would be illustrated in template reconstruction study.

Major Findings:

(1) Twenty-five normal brains have been embedded in jeltrate after marking the commissures and preparing for section in the transverse plane and horizontal plane with reference to the intercommissural line and the plane formed by this line and one perpendicular to it passing through the center of the anterior commissure perpendicular to the mid sagittal plane. All this material has been cut at 2 mm slices and photographed. Positive acetate prints are on file of sections selected at 5 mm intervals throughout the material.

(2) Section of the superior cerebellar peduncle has been carried out in 2 macaque monkeys and one chimpanzee and the resulting degeneration stained by the Nauta-Gygax method. The object of the study was to determine the upper border of the nucleus ventralis lateralis in the primate thalamus. Reconstruction of the first specimen has shown degenerating fibers extending well forward, probably into the posterior portion of the ventralis anterior as well as the ventralis lateralis. In the anterior portion the degenerating fibers may reach as high as the junction of the upper middle thirds of the thalamus height. Posteriorly these do not exceed the lower half of the thalamus.

(3) Template reconstructions of a chimpanzee brain prepared with Marchi method after section of the superior cerebellar peduncle, demonstrated heavy Marchi degeneration only in the lower half of the thalamus. Fine material, possibly pseudo-Marchi material, was present in the upper portion of the thalamus. (Specimen loaned by Dr. A. E. Walker)

(4) The work of serially sectioning human brains both normal and with focal lesions has continued. The serial celloidin sections have been stained with Nissl and Heidenhain methods.

(5) All of the normal material, both for the macroscopic and microscopic series, has now been collected.

(6) A brain with a bilateral parasagittal lesion, which was mentioned in the past year as being urgently needed to complete the focal cortical lesion, has been acquired and is presently in preparation.

Proposed Course of Project:

(1) By means of superimposition tracings of the acetate positive prints, the macroscopic variations of the human thalamus basal ganglion brain stem can be determined.

(2) To define the upper limits of the dentato-rubero-cortical thalamic relay nucleus. The two remaining superior cerebellar peduncle lesions will

be reconstructed. It is planned to carry out in the same chimpanzee a resection of the right precentral cortex, then 90 days later a section of the right superior cerebellar peduncle followed by killing the animal in 10 days. The right thalamus will be prepared in Nissl and Heidenhain series in celloidin to trace retrograde cell degeneration following removal of the motor strip. The left thalamus will be prepared by Nauta-Gygax method to trace the degeneration from the superior cerebellar peduncle lesion. It is hoped that in the same preparation to demonstrate the extent of retrograde cell change following motor cortex ablation and the fiber degeneration following superior cerebellar peduncle lesion.

(3) Once the topography of the nucleus ventralis lateralis can be decided, then the normal nuclear outlines in the five microscopic normal series will be laid out in template reconstructions. Superimpositions of the reconstructions will demonstrate the variation in microscopic anatomy of the human thalamus.

(4) Once a workable cytologic division of the human thalamus can be achieved, then reconstruction of the thalami, after focal cortical lesions to demonstrate the thalamo cortical relations, will be carried out.

Significance to Bio-Medical Research and the Program of the Institute:

A satisfactory variation atlas, even of gross relationships of the thalamus basal ganglia and upper brain stem, is not presently available. No serious study has even been attempted of the microscopic thalamic variation. Knowledge of these relationships is essential for the meaningful plotting of human subcortical electrode placements and lesions.

It was pointed out the year before that this project is now well formulated and ready for intensive work. The present lack of space precludes the possibility of any local collaborator at a professional level. This poses a severe handicap to the steady progress of the work.

Part B included: Yes No

Serial No: NDB(I)-55 SN/OC 200(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Involuntary Movements

Principal Investigator: J. M. Van Buren, M. D.

Other Investigators: C. Ajmone-Marsan, M. D., Herbert Lansdell, Ph. D., Choh-luh Li, M. D., Ayub K. Ommaya, F.R.C.S., H. Enger Rosvold, M. D.

Cooperating Units: Branch of EEG, NINDB; Section on Clinical Psychology, Surgical Neurology, NINDB; Section on Neuropsychology, NIMH

Man Years (computed for the 12 month period)

Total:	2.6
Professional:	1.5
Other:	1.1

Project Description:

Proposed Course of Project: Sub-projects presently under consideration:

(1) The fronto-striate arrest response in man has now been studied in 30 individuals. This response is characterized by an inability to carry out involuntary movement including speech, repetitive movement of the hands or maintained posture of the tongue (extending the tongue). It has been obtained in the left hemisphere in 18 individuals and in the right in 12. The response is relatively high threshold having a mean stimulus threshold of 8.0 ma (2.5 milliseconds, 60 per second unidirectional square pulses). Ancillary phenomena consists of objective mood change, impaired auditory recall during stimulation, post stimulation confusion and spontaneous inappropriate speech with amnesia, contraversive movement of the eyes and head. Eleven patients were tested by continuous performance tests, testing during stimulation with scalp EEG monitoring. In no instance did the scalp recording show evidence of seizure activity dispite the presence of arrest. The six minute testing period was divided into three epochs. The first two minute period showed the severest incapacity due to stimulation and dispite a constant rate of stimulation and constant threshold throughout the remainder of the test, scoring improved with time. The apparent rise in threshold did not appear to be due to tissue change because after a period of 5 to 10 minutes rest, the previous low threshold and characteristic arrest response

is again obtained. The mechanism of this change remains obscure. This experience is presently being collected for publication (with Dr. Li and Dr. George Ojemann).

(2) Alterations in the H reflex with thalamo tegmental stimulation and coagulation. This project has been held up by the absence of the biphasic square wave, monitored constant current, isolated stimulator for which a work request was submitted in September of 1961. The instrumentis presently nearing completion.

(3) Evaluation of motor function in dyskinesia by means of visuo-motor tracking tasks. The equipment necessary to carry out quantitative measurement of the discrepances between the patient's performance and visual input has presently been completed. The original agreement was with Dr. Stark at MIT to provide the pseudo random tracking signal and the machine Fourier analysis. This has been terminated since Dr. Stark is leaving the department. In December 1964, Mr. Cohen undertook to set up a program for local production of the pseudo random input and its analysis.

(4) Equipment for recording extracellular unit activity in the human thalamus and cortex has been completed and is working satisfactorily. Simultaneous units have been satisfactorily recorded both in the thalamus and the motor cortex in the same patient on two instances, and satisfactory records at one or the other site have been obtained in another 10 (See details in Dr. Choh-luh Li's annual report).

(5) EEG's studies employing depth electrodes: Collection of material continues document:

i. The effect of lesions in the basal ganglia and thalamus upon the EEG.

ii. Comparison of depth and scalp activity during normal and induced sleep (with Dr. Ajmone-Marsan).

(6) Study of evoked activity from peripheral stimuli both upon the unit activity in man and upon EEG and the gross implanted electrode recordings, has now become a practical possibility. A program for summing 50 to 100 evoked responses to eliminate random activity has been developed as well as tactal, auditory, and visual stimuli with synching pulses have been developed. The work will probably be recorded on tape and be submitted later for averaging on the Linc Computer.

At the moment, particular interest is centered on the study of secondary sensory afferent impulses and their response to degrees of sleep and barbiturates.

(7) The effect of thalamic lesions upon psychological tests immediately after coagulation and after one year follow-up. Collection of data in this group continues (with Dr. Lansdell).

(8) Topography of pain referrals from stimulation in the fronto temporal dura. The data from this study is presently complete and must be worked up.

(9) With view to better understanding the magnitude of the recordable field with implanted depth electrodes, a study was undertaken to plot the topography of the potential field about the lateral gastrocnemius-soleus nucleus when fired by antidromic shocks. The combined physiological anatomical study is summarized below (with Dr. Karl Frank):

i. The lateral gastrocnemius nucleus in the cat has been investigated by retrograde cell change and found to lie predominately within the L_7 segment of the spinal cord and measure at a maximum about 10.0 x 0.6 x 0.8 mm. In the cell group occupied by the nucleus, two predominant sizes of neurons were found having peak incidences of minimum diameter at 20μ and 40-50μ. The density of the smaller cells was about 399/mm^3 and of the larger about 630/mm^3.

ii. An indirect method of estimation of the population of the lateral gastrocnemius nucleus suggested that the smaller cells in the nucleus (in ratio of about 2 small to 3 large cells) must project to the periphery in order to obtain results comparable to counts made directly of the cells showing retrograde chromatolysis. It is suggested that this is the motor supply to the intrafusal fibers.

iii. Golgi studies of the cells in the region of the lateral gastrocnemius nucleus showed that they had a diffusely radiating dendritic pattern in the transverse plane (up to 1500 to 2000μ in diameter) but when viewed in horizontal section the dendrites tended to show a transverse orientation.

iv. The antidromic response to stimulation of the lateral gastrocnemius nerve showed variation in the number of participating elements varying from a single cell to several with relatively little change in the magnitude of the recordable potential field. The latter approximated the size of the dendritic arborization of the larger cells.

v. The gradient of the initial positive deflection with distance (ascribed to activity within the myelinated segment of the axon) was less than that of the major negative deflection (AB spike) of the antidromic action potential.

vi. The gradient of the AB spike when recorded with a 2-4μ pipette in its "normal" negative going form, was quite similar to its "injured" positive form recorded with large electrodes.

Part B included: Yes <u>No</u>

Serial No. NDB(I)-60 SN/CN 706(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964, through June 30, 1965

Part A.

Project Title: Clinical, Biochemical and Genetical Studies of Mental Retardation, Progressive Cerebral Degeneration and Cerebral Palsy in Children

Principal Investigator: Anatole Dekaban, M.D., PhD.

Other Investigators: William Moore, M.D. and Owen Rennert, M.D.

Cooperative Units: District of Columbia Children's Center, Laurel, Maryland

Man Years (July 1, 1964, through June 30, 1965)
Total: 2.05
Professional: 1.35
Other: 0.7

Project Description:

Objectives: Actually this is a large permanent program. Chronic neurological disorders affecting children constitute one of the major medical and social problems of our times. According to conservative estimates for this country, about four million children are currently and permanently handicapped by mental retardation, cerebral palsy, epilepsy or progressive cerebral degeneration. Many of them have to be cared for to the end of their life by the joined services of the society and government. Until recently these conditions did not receive sufficient attention in the field of medical research. Our main objectives are:

1. To subdivide the large number of studied patients with mental retardation and cerebral palsy and epilepsy into distinct clinical categories using developmental and neurological examinations along with laboratory procedures.

2. To establish types of cerebral lesion characteristic of each category whenever possible.

3. To apply basic biochemical and genetic methods to selected diseases or syndromes within each category with the aim of advancing the underlying pathogenesis and etiology.

4. To advance when possible the therapy and prevention of these chronic diseases.

Methods Employed:

1. Detailed general medical and neurological studies.
2. Developmental and psychological examinations.
3. Modified electroencephalographic studies.
4. Biochemical asseys of protein, lipid and carbohydrate metabolism.
5. Cytogenetic and pedigree studies.
6. Employment of special procedures such as pneumoencephalography and analysis of brain biopsies whenever these are performed for diagnostic purposes.

Patient Material: 45 inpatients and 56 outpatients including consultations.

Clinical Project

Major Findings:

1. A group of 10 patients suffering from Hurler's syndrome and their immediate families are currently under study. Contrary to general belief our investigation indicates that the autosomal recessive and sex-linked variety cannot be distinguished by the type of muco-polysaccharides excreted. Using electrophoresis in several buffers we have demonstrated presence of heparitin monosulphate and chondroitin sulphate B in the members of the families with either mode of inheritance. There are, however, other metabolites which will probably make it possible to identify heterozygotic members of the family. This aspect is being studied intensively at the present time.

2. Inheritance of familial type of mental deficiency has been studied using clinical, laboratory and pedigree methods. A group of families was separated where the mode of transmission of the abnormal trait was of an autosomal dominant type. The fact that rearing of a proportion of offspring in good foster homes since birth did not influence the expressivity of the trait nor did it change significantly the general range of the intelligence quotient permitted to draw certain conclusions pertaining to the patho-genesis of the condition. Another observation which was derived from this investigation permitted formulation of a supposition to be further tested: The mild type of familial mental retardation forms an entity of its own and does not overlap with more severe types of oligophrenia.

3. Certain types of congenital malformations involving the brain of children can be successfully treated by surgical means without a resultant functional impairment. This was demonstrated by the detailed follow-up study of the head-conjoined siamese twins whom we separated eight years ago by using a noval technique in covering large bony and skin defects.

4. A woman who received excessive body irradiation developed a persisting clone of cells with abnormality involving the presumed No. 9 chromosome as well as other chromosomal aberrations which are typical of radiation injury. Recently she gave birth to a grossly malformed child. The child and the mother have been subject to extended metabolic and cytogenetic studies. It is for the first time that an abnormal clone of cells has been demonstrated in a human exposed to excessive ionizing radiation. This finding may have very important genetic implications.

Significance to Bio-Medical Research and the Program of the Institute: This project is a large program in the field of chronic neurological disorders in children. As such it is of necessity broad in scope and requires the application of many clinical and laboratory approaches. Our present state of knowledge in this field is decisively inadequate and categorization of the conditions into groups and individual disease entities is a necessary prerequisite and it has to proceed parallel to the applied and basic laboratory studies.

Proposed Course of the Project: This program covers the field of the most important responsibilities of NINDB and must be expanded in personnel and space if we are to remain in the leading group; it is a permanent program.

Part B included Yes No

PHS-NIH
Individual Project Report
July 1, 1964, through June 30, 1965

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Dekaban, Anatole and Klein, David: Familial Mental Subnormality. Proceedings of the International Congress on the Scientific Study of Mental Retardation, Copenhagen, 1965.

Dekaban, Anatole S.: Metabolic and chromosomal studies in leprechaunism. Arch. Dis. Childh., 1965. In press.

Baldwin, Maitland and Dekaban, Anatole: Cephalopagus twins, seven years after separation. J. Neurosurg., 1965. In press.

Honors and Awards relating to this project:

1. Assistant Professor of Neurology at George Washington University Medical School.

2. Consultant, District of Columbia Children's Hospital.

3. Consultant, District Training School.

Serial No. NDB(I)-60 SN/CN 707(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964, through June 30, 1965

Part A.

Project Title: Study of Pathological Lesions in the Central Nervous System Occurring During Prenatal, Intranatal and Early Postnatal Life

Principal Investigator: Anatole Dekaban, M.D., PhD.

Other Investigator: Rosemarie Thron

Cooperating Units: None

Man Years (July 1, 1964, through June 30, 1965)
Total: 1.0
Professional: 0.3
Other: 0.7

Project Description:

Objectives: In the majority of patients the causation and underlying pathology of mental deficiency and cerebral palsy are largely unknown. Detailed examination of the brains of children who suffered from such disorders for the purpose of correlating these lesions with the clinical data is expected to provide valuable information for etiological clues, thus paving the way for possible preventive measures. Limited, experimental approach in animals is used when essential.

Methods Employed:

1. Detailed evaluation of the clinical and laboratory data of the children who suffered from chronic neurological disorders and subsequently died.

2. Gross examination of brains.

3. Microscopical study of large histological sections stained by a variety of chromatic, myelin and silver methods as well as by special histochemical procedures.

4. Reconstruction of the brain and spinal cord in young human embryos.

5. X-ray radiation of pregnant mice in various stages of pregnancy.

Material: Brains of infants who died with a diagnosis of birth injury, cerebral palsy, epilepsy or mental deficiency. Central nervous system of the offspring of mice whose mothers were x-radiated during pregnancy.

Major Findings:

1. Sixteen human brains from the patients who died of cerebral palsy and mental retardation were processed. Full size coronal sections were utilized and a variety of histological and histochemical staining procedures employed. This has considerably increased our collection on cerebral malformations, birth injuries and progressive degenerative disorders.

2. During the last year a large study on the formal origin of developmental porencephalies and polymicrogyria was completed. The study was based on 11 human brains, cut coronally in large sections at frequent intervals. This permitted reconstruction of the lesions which in turn allowed an analysis of their distribution in relation to arterial cerebral circulation. The following are the results:

   Porencephalies involving the entire thickness of cerebral hemispheres are associated with polymicrogyria in a proportion of cases. Generally, both these lesions are bilateral and symmetrical although they can be unilateral; their size ranges from about 3 sq. cm. to almost complete absence of cerebral convesity. Gross and histological examination of eleven brains with such abnormalities revealed the existence of several patterns of topographical distribution of the lesions. Thus, the gross defects may involve (1) mesial and parasagittal portions of both cerebral hemispheres (2) large portions of the centro-lateral aspects of the hemispheres (3) the entire cerebral convexity may be absent (hydranencephaly) or (4) the defects may be relatively small involving only a portion of cerebral hemispheres. The polymicrogyria is usually situated immediately adjacent to the porus.

   The histological features disclosed indicate that these two lesions originate prior to the sixth month of gestation and that they may be related to a common causative factor. The analysis of topographical distribution of the large pallial defects and of the polymicrogyria in the present series suggests strongly arterial origin of these two lesions. Thus, large defects in the cerebral hemispheres were located in the areas supplied with blood by either anterior, middle or both these arteries and polymicrogyria was present in the adjacent areas belonging to the arterial blood shed. Impairment of fetal systemic circulation in association with such local factors as cerebral edema or variations in the caliber or site of origin of certain arterial branches are probably responsible for the occurrence of these two lesions.

Significance to Bio-Medical Research and the Program of the Institute:
It has been demonstrated that:

1. A proportion of porencephalies originate during fetal life and specifically during 3-5 months of gestation.

2. These porencephalies are surrounded by dysgenetic (polymicrogyric) cortex.

3. The distribution of these lesions is in the territory of the blood supply of major cerebral arteries.

Thus, a considerable light has been shed on the pathogenesis of these structural abnormalities which underlie cerebral palsy and mental retardation. Among others, this permits better formulation of projects on the etiology of certain types of cerebral palsy, mental retardation and epilepsy. Some of the implications are discussed in the article which is currently in press.

Proposed Course of the Project: This project has a wide scope and pertains to one of our major interests. It supplements also our principal project, NINDB-74(c).

Part B included Yes No

PHS-NIH
Individual Project Report
July 1, 1964, through June 30, 1965

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Dekaban, Anatole: Large defects in the cerebral hemispheres associated with cortical dysgenesis. J. Neuropath. Exp. Neurol., Vol. 24, 1965. In press.

Serial No. NDB(I)-63 SN/CN 1026(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964, through June 30, 1965

Part A.

Project Title: Clinical and Biochemical Studies in Epilepsy

Principal Investigator: Anatole Dekaban, M.D., PhD.

Other Investigator: Diane Mizel

Cooperating Units: None

Man Years (July 1, 1964, through June 30, 1965)
Total: 0.9
Professional: 0.3
Other: 0.6

Project Description:

Objectives: Idiopathic epilepsy is a clinical syndrome of unknown etiologies. In the first instance it is important to accumulate a sufficient number of such patients for the purpose of subdividing them into different categories depending on certain clinical and laboratory findings. This will permit better directed therapeutic application of certain regimens and drugs under investigation. The patient's response or its lack will provide us some informations on the pathogenesis of the condition.

Methods Employed:
1. Necessary clinical examinations and tests.
2. Routine and special electroencephalographic studies.
3. Pneumoencephalography and arteriography when indicated.
4. Urinary and plasma amino acids.
5. Asseys of carbohydrate metabolism.
6. Plasma lipid studies.
7. Endocrinological asseys when indicated.
8. Application of special therapeutic procedures.

Patient Material: 14 inpatients and 18 outpatients with idiopathic epilepsy.

Clinical Project

Major Findings:
1. Ten suitable children with idiopathic epilepsy were established on the ketogenic diet and detailed studies of their lipid profiles

were conducted. It was found that the average values for the total lipids, total cholesterol and total ketones in the plasma of these children increased significantly ($P<0.01$) by the tenth day of the high fat diet as compared to the values when they were fed the normal diet. The increase in plasma phospholipids and triglycerides was less striking ($P<0.05$); the total and free fatty acids did not change although this might have been related to the high individual variations. Plasma glucose levels were consistently lower ($P<0.05$) when the children were fed the high fat diet.

2. A group of children who were initially considered to have epilepsy were found to be suffering from hypoglycemia of childhood of l-leucine sensitive variety. Extended hormonal and dietary investigations as well as the therapeutic approaches were conducted. A new type of dietary regimen was finally devised which proved superior to the previously used methods of treatment of this condition. Principally, the amount of dietary l-leucine permitted per day is strictly controlled, the food is administered in 7 divided portions and the principal feedings with protein are followed 30 minutes later by 20-30 gm. of carbohydrates. The details of this approach are prepared for publication

Significance to Bio-Medical Research and the Program of the Institute: As it has been demonstrated by our previous findings (J. Dis. Children, 100:181-188, 1960) frequent daily attacks (usually of minor variety) if occurring during the first years of life may lead to irreversible mental retardation. So far, the broad biochemical approach to this type of epilepsy in children has been scarcely used. Some of our previous publications indicate that such studies can be fruitful. This investigation extends the scope of the study of epilepsy which is the major theme of the Surgical Neurology Branch, NINDB

Proposed Course of the Project: The project will continue for a number of years to come.

Part B included Yes No

PHS-NIH
Individual Project Report
July 1, 1964, through June 30, 1965

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Dekaban, Anatole and Mizel, Diane: Plasma lipids, glucose and ketones in young children on a high fat diet. Amer. J. Clin. Nutr. 15:358-364, December, 1964.

Serial No. NDB(I)-60 SN/CN 708(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964, through June 30, 1965

Part A.

Project Title: Cytogenetical Study of Human Chromosomes Especially in Patients with Congenital Malformations and Mongoloids and Their Families

Principal Investigator: Anatole Dekaban, M.D., PhD.

Other Investigators: Jan Steusing

Cooperating Units: Michael Bender, PhD., Biological Laboratory, AEC, Oak Ridge, Tennessee

Man Years (July 1, 1964, through June 30, 1965)
Total: 1.1
Professional: 0.3
Other: 0.8

Project Description:

Objectives: Recently it has been found that a proportion of patients with severe congenital malformations and also mongoloids have abnormal chromosomal constitution. Since chromosomes contain hereditary material responsible for expression of all traits, the abnormality in the number of chromosomes or their morphology may be associated with or even be a cause of malformation. This approach is opening a new horizon in the research on congenital malformations and certain familial disorders.

Methods Employed:

1. Comprehensive clinical and laboratory investigation of selected patients to establish diagnosis and degree of abnormality.

2. Tissue culture of the peripheral blood after red blood cells are removed. Bacto-Phytohemagglutinin is added in order to stimulate proliferation of the white blood cells. Six hours prior to sacrificing the culture colchicine is added in a final concentration of 0.5×10^{-6}.

3. Through a number of steps the white blood cell suspension is centrifuged and washed, then exposed to hypotonic salt solution and fixed.

4. Concentrated suspension of cells are stained on the slide with orcein, Giemsa or Faulgen methods.

5. Under high power resolution in light and contrast phase microscope the chromosomes are counted, their morphology studied and karyotype figures made for final analysis.

6. When indicated, using tritiated thymidine, DNA duplication sequence is studied in the chromosomes during the late phase of leukocyte tissue culture.

Material: Patients with congenital malformations, mongoloids, and certain metabolic disorders as well as their families are the subject of this study.

Major Findings:

1. Karyotypic examination was carried on 42 of our patients and 78 of their immediate families. In addition, limited service was provided in performing cytogenetic analysis on 7 patients from other NIH services. Nineteen percent of the subjects studied showed chromosomal abnormalities; these were of diagnostic importance and also provided research material which is currently evaluated.

2. Large cytogenetic study on 92 mongoloids and their parents is in the initial stage of evaluation. This study is an extension of our preliminary report based on 14 mongoloids and their families published in Cytogenetics 2:61-75, 1963. Our main proposition tested is that the incidence of chromosomal aberrations in the families of mongoloids (even if translocation trisomy is not considered) is higher than in normal population. If it is so, this may mean that these aberrations may predispose to nondisjunction during gametogenesis.

3. Detailed study was undertaken of the set of female twins, one of whom is a mongoloid and has 48 chromosomes, while the other is normal and has 46 chromosomes. Despite the fact that they are discordant for the trait of mongolism, we have proven them to be identical twins by blood groups, protein types and palm prints. Chromosome studies included the whole family and employment in addition to blood cultures of the skin culture and DNA duplication studies using tritiated thymidine. It is considered that the original zygote was a 47 chromosome mongoloid. The findings have important implications for human cytogenetics in general.

Significance to Bio-Medical Research and the Program of the Institute: The etiology and pathogenesis of a vast number of conditions associated with congenital malformation and mental deficiency are largely unknown. Demonstration of chromosomal aberration in some of these patients or their parents is a great step forward in our

understanding of these conditions and it may even suggest in the distant future certain preventive measures. Clinical and experimental study of x-radiation on the offspring and chromosome constitution are of great importance in the present era.

Proposed Course of the Project: This is a long term project. The findings are clearly applicable to our principal projects relating to the broad field of mental retardation and congenital malformations.

Part B included Yes No

PHS-NIH
Individual Project Report
July 1, 1964, through June 30, 1965

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Dekaban, A. S.: Twins, probably monozygotic: one mongoloid with 48 chromosomes, the other normal. Cytogenetics, 1965. In press.

Serial No. NDB(I)-60 SN/CN 709(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964, through June 30, 1965

Part A.

Project Title: Cranial Measurements and Internal and External Orbital Indices in Males and Females from Birth to Maturity

Principal Investigator: Anatole S. Dekaban, M.D., PhD.

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1964, through June 30, 1965)
Total: 0.15
Professional: 0.15
Other:

Project Description:

Objectives: The purpose of this project is to supply averages and standard deviations of cranial measurements during early childhood. Although several series with progressive measurements of many diameters of the skull exists, certain dimensions especially cranial capacity and inter-orbital distance have been lacking. In our overall program on neurological abnormalities in children the last mentioned dimensions were needed.

Methods Employed:
1. Anthropological measurements of the human dried skulls.
2. X-ray measurements of these skulls.
3. Physical anthropological measurements of the heads in children.
4. X-ray measurements of a random sample from these subjects.
5. Statistical analysis in various age horizons.

Material: 28 dried human skulls
373 children

Major Findings: Averages and standard deviations of 7 cranial and facial dimensions and 5 indexes were calculated from the measurements in 178 boys and 195 girls and the data tabulated and used in our book on "Neurology of Infancy" published by Williams and Wilkins. This table and the table with the data on measurements in older children are in routine use by the clinical staff of our Section. Calculation of cranial capacity of 28 dried skulls from anthropological and x-ray

dimension was made and these were validated against actual measurement of the volume of these skulls. The results obtained provided the basis for devising appropriate formulae in calculating cranial capacity in living subjects.

Significance to Neurological Research: Provision of methods for calculation of cranial capacity and interorbital distance has added to our assessment of conditions associated with cranial deformities, increased or reduced sizes of the head and hypertelorism. The tables of normal values in young children of both sexes permit easy use of the methods. It is known that mental retardation and certain neurological disorders can be associated with the listed above clinical conditions.

Proposed Course of Project: This project is completed.

Part B included Yes No

PHS-NIH
Individual Project Report
July 1, 1964, through June 30, 1965

Part B. Honors, Awards and Publications

Publications other than abstracts from this project

Dekaban, A. and Lieberman, J. E.: Calculation of cranial capacity from linear dimensions. Anat. Rec. 150:215-219, November, 1964.

Serial No. NDB(I)-65 SN/CN 1204(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland
New Project

PHS-NIH
Individual Project Report
July 1, 1964, through June 30, 1965

Part A.

Project Title: Studies On the Incorporation of an Amino Acid Analogue - TRIFLUOROLEUCINE - Into Protein of Mammals

Principal Investigator: Owen M. Rennert, M.D.

Other Investigator: Anatole S. Dekaban, M.D.

Cooperating Units: None

Man Years (July 1, 1964, through June 30, 1965)
Total: 0.8
Professional: 0.6
Other: 0.2

Project Description:

Objectives: Trifluoroleucine is an analogue amino acid which can be incorporated into new proteins under certain conditions. The changed protein has different physiological and biochemical properties. It has been shown before by one of us that the analogue administration ameliorates significantly the course of leukemia in mice. Our aim is to study the basics of protein synthesis in mammalian embryos using the analogue.

Methods Employed:
1. Standard methods of amino acid analysis.
2. Electrophoresis and diffusion studies.
3. Ultracentrifugation - density gradients.
4. Tracer studies.
5. Histochemical studies.

Material: Synthesized by us amino acid analogue trifluoroleucine. Mice as needed.

Major Findings: This is a new project.

Significance to Bio-Medical Research and the Program of the Institute: Our preliminary study indicates that the in vivo incorporation of trifluoroleucine can be achieved in embryonic systems. Long term aims relate to defining in chemical terms the protein synthesizing

systems of embryonic tissue, and to correlate changes in the primary structure of the proteins (and secondary) with morphogenesis.

Proposed Course of the Project: This project has just begun. Its anticipated duration is two years.

Part B included Yes No

Serial No. NDB (I) - 63 SN/NP 1027 (c)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Changes in chloride and water content of brain tissue after chemical injury of the blood-brain barrier.

Principal Investigator: Oskar Steinwall, M.D.

Other Investigators: Eugene Streicher, Ph.D., Igor Klatzo, M.D.

Man Years (computed for the 12 month period)

Total:	.8
Professional:	.9
Other:	1.7

Project Description:

By means of standardized method for intracarotid injection of injurious solutes in rabbits blood-brain barrier (BBB) damage, characterized by the abnormal extravasation of acid dyes was produced within one hemisphere, the other serving as a control. Comparative assay of the chloride and water content in damaged and undamaged hemisphere of the same brain was performed varying the type and concentration of the injuring solute and the time interval between injury and the termination of the experiment.

Method Employed:

Adult rabbits under urethane anesthesia were used. The unilateral BBB injury was produced by injection of chemicals with known BBB damaging properties via a catheter placed in the left common carotid artery (after ligation of its external branches) with a pressure causing the expulsion of the blood in the left hemisphere (controlled by visual inspection of the cortex through the dura via a trephine opening). The injection time was usually 30 sec. Afterwards the injected hemisphere

was recirculated with blood from the circle of Willis. The degree and extent of a BBB damage was assessed by intravenous injection of various tracer dyes. The rabbits were sacrificed by exsanguination at various time intervals after the carotid injection and symmetrical samples from the injected (left) and the control (right) hemispheres were taken for chloride and for water determinations.

In most experiments the injected damaging agent was $HgCl_2$ (0.01 - 01. mM) while penicillin G was administered in a smaller group of animals. Control experiments were carried out with intracarotid injections of Ringer's solution.

Major Findings:

A comparison between the injected (barrier-damaged) and the non-injected (control) hemispheres with regard to the chloride and water content revealed the following results:

1. Control experiments (6 rabbits injected with Ringer's solution; 8 rabbits injected with chemicals but at a dose not producing any microscopic evidence of extravasation of tracer dyes) showed either no significant differences between hemispheres in chloride and water content or slightly higher values in the injected side (up to 1.5 mEq/Kgm chloride and 0.35% water).

2. Experiments with slight or moderate BBB damage induced by mercuric chloride (16 rabbits) showed consistently a lower chloride content on the barrier damaged side than in the control hemisphere. The differences exceeded 2 mEq/Kgm in 10 rabbits and 4 mEq/Kgm in 3 rabbits. The side differences in water content were on the whole not significant.

3. Experiments with slight or moderate BBB damage induced by penicillin G (5 rabbits) showed higher chloride (0.8 - 3.0 mEq/Kgm) content on the BBB damaged hemisphere. With regard to water content there were no significant differences.

4. Experiments with strong mercurial BBB damage (7 rabbits) showed a considerable increase of both chloride (4 - 10 mEq/Kgm) and water (0.62 - 4.22%) in the damaged hemisphere.

In interpretation of these findings the most interesting observation is the effect of slight or moderate mercurial BBB damage expressed in significantly decreased chloride content in the hemisphere showing extravascular passage of tracer dyes. This suggests a functional disturbance in exchange mechanisms operating at the blood-brain interphase and not a mere leakage through ruptured BBB structures. On the other hand, stronger mercurial injury resulted in the development of edema (evident grossly and microscopically) which as usual is associated with an increase in both chloride and water content. The effect of

penicillin is of a more complex nature since this substance after passing through the blood vessels appears to penetrate quickly to the neurons as it is evidenced by development of convulsive phenomena predominantly on the side contralateral to the injection.

Proposed course of the project: This project has been temporarily discontinued due to the departure of the principal investigator. It is planned to conclude these investigations at the University of Goteborg during July-August, 1965.

Part B included No

Serial No. NDB (I) - 63 SN/NP 1028 (c)
1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Experimental Production of Neurofibrillary Degeneration

Principal Investigator: Igor Klatzo, M.D.

Other Investigators: Henryk Wisniewski, M.D.
Eugene Streicher, Ph.D., Robert M. Terry, M.D.

Cooperating Units: Albert Einstein College of Medicine

Man Years (computed for the 12 month period)

Total:	1.7
Professional	.7
Other:	1.0

Project Description:

This investigation was concerned with the experimental production of neurofibrillary degeneration by intracerebral or intrathecal injection of minute amounts of aluminium phosphate in rabbits. This experimentally produced alteration may serve in some respects as a model for a study of the neurofibrillary changes which are observed in a number of neurological degenerative conditions in man such as e.g. Alzheimer's disease and senile dementias.

Methods Employed:

0.1 ml of aluminium phosphate, prepared as the Holt's adjuvant, was injected through a burr hole into one of the hemispheres in rabbits. The animals were sacrificed at various time intervals. The brain and spinal cord tissue was processed for light and electron microscopy as well as for the determination of the wet/dry weight ratios and of the thiocyanate space.

Major Findings:

After a latent period of one week or longer the $AlPO_4$ injected rabbits developed ataxia followed by a rapid deterioration of their general condition. Observations under the light microscope revealed the presence of striking neurofibrillary tangles in the larger neurons of the brain stem, the medulla and the spinal cord. These changes were particularly conspicuous in polarized light. Electron micrographs of neurons from the corresponding areas showed striking focal clearings of the cytoplasm which were filled with a dense array of more or less parallel filaments resembling Alzheimer's neurofibrillary tangles. The wet/dry weight ratios of the brain areas affected by the neurofibrillary degeneration were within the normal limits. On the other hand, these areas showed an increase in the thiocyanate space.

Proposed course of the project:

This project is completed. The results are presented in the paper "Experimental Production of Neurofibrillary Degeneration 1. Light Microscopic Observations", which will appear in the J. NEUROPATH. EXP. NEUROL.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Klatzo, I., Wisniewski, H., and Streicher, E.: Experimental production of neurofibrillary degeneration 1. Light microscopic observations. J. Neuropath. Exp. Neurol. 24: 183-199, April, 1965.

Serial No. NDB (I) - 63 SN/NP 1030 (c)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Observations on Differential Penetrability of Simultaneously Administered Tracers in Blood-Brain Barrier (BBB) Damage.

Principal Investigator: Oskar Steinwall, M.D.

Other Investigators: Igor Klatzo, M.D., Diane E. Smith, M.S.

Man Years (computed for the 12 month period)
Total: 1.6
Professional: 1.2
Other: .4

Project Description:

In view of the recent concept suggesting that the BBB phenomenon may be related to a variety of specific transfer mechanisms, a selectively differential behaviour of the BBB was studied in chemical injuries produced by intracarotid injection of various compounds.

Methods Employed:

Unilateral BBB injury was produced by intracarotid injection of mercuric chloride (0.03 - 0.08 mM), penicillin (3.5 - 5%), or sodium acetrizoate (14-18%). This was followed by systemic administration of combination of two different fluorescent and radioactive tracers. The combinations of tracers were as follows:

a) red-fluorescent Rhodamine B labeled albumin (FLA) with green-fluorescent fluorescein labeled albumin (FLA).

b) red fluorescent Evans Blue - ablumin complex (EBA) with FLA

c) EBA with green fluorescent fluorescein labeled gamma globulin FLGG

d) RLA with FLGG

e) sodium fluorescein or FLA with C^{14} sucrose

f) sodium fluorescein or FLA with C^{14} inulin

g) sodium fluorescein with C^{14} methyl-o-glucose

The brain tissue was studied by fluorescence microscopy and radioautography.

Major Findings:

Combinations of red and green fluorescent albumins showed no separation in the distribution pattern on the damaged side. Simultaneous administration of red fluorescent albumins and green fluorescent globulins revealed in numerous instances a distinct separation in distribution of protein tracers on the damaged side. In slightly or moderately damaged areas numerous blood vessels were surrounded by only red fluorescence. The perivascular exsudates and globules showed, a range of color depending on relative concentration of respective tracers. Differential passage from the injured vessels was also observed in combinations of fluorescent and radioactive tracers. C^{14} inulin appeared to penetrate in damaged areas more extensively than sodium fluorescein or FLA. An interesting result was obtain in sodium fluorescein - C^{14} methyl-o-glucose combination. Whereas in moderate or severe BBB damage both sodium fluorescein and C^{14} methyl-o-glucose spread intensely from the injured vessels, in very slight BBB damage in which no abnormal passage of sodium fluorescein could be detected, there was a distinct inhibition of the normal transport of methyl-o-glucose from the blood into the brain tissue.

Proposed course of the project:

This project is basically concluded. It demonstrated conclusively the selective features in vulnerability of the BBB. The inhibition of normal glucose transfer from blood to brain in very slight BBB injuries, which are undetectable by visual tracers, may be of considerable clinical significance.

Part B included No

Serial No. NDB (I) - 63 SN/NP 1031 (c)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Observations on the Blood-Brain Barrier and the CSF Pathways in Sharks.

Principal Investigator: Igor Klatzo, M.D.

Other Investigators: Oskar Steinwall, M.D.

Man Years (computed for the 12 month period)
Total: 1.0
Professional: .6
Other: .4

Project Description:

In view of the special features of CSF production and CSF-blood exchanges elucidated previously in sharks by Drs. D. P. Rall and C. G. Zubrod, as well as, in view of the striking morphological relationship of glia to the blood vessels and the pia (observed by I.K.) the present study was conducted in order to investigate the morphological aspects of the CSF pathways and the basic behaviour of the blood-brain barrier (BBB) in this species.

Methods Employed:

Lemon and nurse sharks provided by the Lerner Marine Laboratory, American Museum of Natural History, Bimini, Bahamas were used. The Sharks were anaesthesized with MS-222 and placed on the operating table with sea-water circulating through the gills. Several types of brain and cerebellum lesions were produced by the application of cold, heat and chemical substances. The animals received intravenous injection of the Evans Blue tracer before the operation. Following the brain injury the sharks were returned to the net-enclosures at the dock and

subsequently sacrificed at various time intervals. In several sharks an attempt of breaking the BBB was made by injection of mercury chloride into the arteries supplying the brain. In another series of sharks Evans Blue was introduced into the third ventricle and the curarized animals were sacrificed after 1 hour. The brain tissue in all animals was fixed by intravascular perfusion with formalin, formalin-ammonium bromide and glutaraldehyde (for electron microscopy).

Major Findings:

The sharks subjected to various lesions revealed a striking resistance of the brain tissue to an injury. The lemon shark sacrificed 3 days after heat injury showed a deep, crater-shaped defect of the brain and cerebellar tissue. However, the surrounding nervous tissue showed no evidence of either edema or extravasation of the Evans Blue tracer. Histologically, the zone surrounding the injury showed only a minimal reaction. Similar resistance to brain injury was observed in sharks sacrificed at earlier stages. The sharks injected with mercury chloride and Evans Blue revealed a striking resistance of the cerebral vasculature to the mercury chloride. Although the dose of $HgCl_2$ exceeded that used in rabbits for disruption of the BBB, in sharks there was no evidence of extensive parenchymatous extravasation of the tracer as it is regularly observed in the rabbits. The alteration of the BBB was demonstrable only in the intense staining of the vascular walls with the Evans Blue tracer.

The sharks which were subjected to the intraventricular infusion of the Evans Blue revealed the passage of this tracer into the whole ventricular system. In addition there was visible penetration of the tracer into the saccus vasculosus, into the system of channels crossing the cerebellum and into the ventricular outpouchings along the olfactory nerves. There was no passage of the tracer into the "subarachnoidal" spaces surrounding the brain and the cerebellum.

Proposed course of the project:

This project is completed and submitted for publication (ACTA NEUROPATHOLOGICA, in press).

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Klatzo, I., Steinwall, O.: Observations on the blood-brain barrier and the CSF pathways in sharks. Acta Neuropath. (In press).

Serial No. NDB(I)-64 SW/NP 1207(c)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Effect of Systolic Blood Pressure on the Dynamics of Brain Edema.

Principal Investigator: Igor Klatzo, M.D.

Other Investigators: Diane E. Smith, M.S., Ayub K. Ommaya, M.D.

Man Years (computed for the 12 month period)

Total:	1.3
Professional:	.9
Other:	.4

Project Description:

This investigation is concerned with the effect of systolic blood pressure on the dynamics of brain edema. The blood pressure in cats was altered by means of hyper - and hypotensive drugs administered prior to the production of brain edema. Edema was produced by the application of a cooled metal plate to the exposed cerebral cortex. Changes of the systolic blood pressure significantly affected the rate of progression of edema through the white matter of the injured gyrus. It was shown that the lowering of the blood pressure can almost completely prevent the development of edema.

Methods Employed:

Blood pressure was recorded through polyethelene catheters placed in the femoral artery under nembutal anaesthesia. The elevation of the blood pressure was produced by the intravenous drip of Levophed (2 ml of a 0.2% solution added to 500 ml of 5% Dextrose). The lowering of the blood pressure was obtained by intravenous drip of Arforad (10 ml of a 0.5% solution added to 500 ml of 5% Dextrose). After stabilizing the blood pressure at a desired level, edema was produced by means of a cold plate (-56°C) applied to the previously exposed cerebral cortex for one minute.

As a blood-brain barrier tracer 3 ml of 10% sodium fluorescein was injected prior to the application of the cold plate. The animals were sacrificed at varying time intervals and the brains were sectioned for gross observations and photography under the U. V. light.

Major Findings:

Elevation of the systolic blood pressure up to 200 mmHg resulted in a striking acceleration of the progression of the edema. Thus, the extent of the edematous area in cats with 200 B.P. sacrificed after 2 hours following cold lesion corresponded to that observed in animals under normal blood pressure, 10 hrs. after cold injury. Conversely, cats with systolic B. P. lowered down to 50 mmHg which were sacrificed 6 hrs. following the cold injury revealed a conspicuous inhibition in the spread of edema, the area outlined by the fluorescein tracer corresponding to that observed in normotensive cats 30 minutes after cold injury.

Proposed course of the project:

Further investigations concerning the effect of the systolic B.P. on the development of the brain edema will be concerned with the relationship between the duration of altered B.P. and the dynamics of edema. Particularly, an attempt will be made in assessment of the value of hypotension in controlling the course of edema, which might be of a considerable clinical significance.

Part B included No

Serial No. NDB(I)-61 SN/OC 802(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Single Cell Discharges in Motor Cortex in Response to Stimulation of Basal Ganglia and Cerebellum.

Principal Investigators: Choh-luh Li, M. D., Ph.D. and Maitland Baldwin, M. D.

Other Investigators: J. Clayton Davie, M. D. and John M. Tew, Jr., M. D.

Cooperating Unit: None

Man Years (Computed for the 12 month period)
Total: 2.0
Professional: 1.5
Other: 0.5

Project Description:

Objective: To establish physiological connections between the so-called extra-pyramidal and pyramidal systems and to study their physiological importance on motor activity.

Methods Employed: The spike activity from the motor cortex in response to stimulation of various subcortical and cerebellar structures is recorded on motion picture film as well as on magnetic tapes and electronic digital recorders. Cats either subjected to barbiturate or mid-brain transection are used.

Major Findings: Synaptic pathways were found to exist between the motor cortex and the following structures: the putamen, globus pallidus, zona incerta, field of Forel, substantia nigra, red nucleus, dentate nucleus and interposital nucleus. Of particular interest are the bilateral projections from the cerebellar nuclei to the motor cortex.

Significance to Bio-Medical Research: The results of this investigation suggest that pathways from the various "extra-pyramidal" structures may not necessarily involve the nucleus ventralis lateralis of the thalamus.

This is particularly true for the cerebello-cortical connection. Further, the long lasting facilitatory and inhibitory effects of the cerebellum upon single cell discharges from the ipsilateral and contralateral motor cortex further emphasize the importance of the cerebellum in motor function.

Proposed Course of Project: This is to be extended to the study of physiological connection of the cerebellum and various subcortical structures.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

Li, C-L., and Davie, J. C.: Evidence of afferent fibers in the motor cortex from subcortical basal nuclei. J. Neurosurg. (in press).

Honors and Awards relating to this project:

Serial No. NDB(I)-62 SN/OC 913(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Single Cell Discharges from Cerebral Cortex of Man

Principal Investigator: Choh-luh Li, M. D., Ph.D.

Other Investigators: John M. Van Buren, M. D. and
John M. Tew, Jr., M. D.

Cooperating Unit: Instrument Engineering & Development Branch, NIH, DRS

Man Years (Computed for the 12 month period)

Total:	3.0
Professional:	2.5
Other:	0.5

Project Description:

Objective: This investigation concerns the discharge pattern of cells in the cerebral cortex and thalamus in relation to epileptic activity and involuntary movements. The ultimate aim is to elucidate the physiological mechanisms by which these neurological disorders are produced.

Methods Employed: These were briefly described in a previous report. In addition, electromyography from flexor and extensor muscles and accelerometry are simultaneously obtained with microelectrode recordings from the cortex and thalamus. Cross and auto-correlations are studied by means of a Link computer based on the data recorded on the multi-channel magnetic tape recorder.

Major Findings: It was found that (A) in the epileptic cortex there are at least two types of cells: 1. those which discharge synchronously with the epileptic waves and 2. those which discharge independently of the epileptic waves. The significance of these two types of cells upon the mechanism of epileptic activity will be reported at a later date after all the data are analyzed by the computer. (B) In the motor cortex and thalamus of patients with involuntary movements, there are at least 5 types of cells: 1. those which discharge only with contraction of certain groups of muscles, 2. those whose discharges are increased with contraction of certain groups

of muscles, 3. those which discharge only with relaxation of certain groups of muscles, 4. those whose discharges are decreased with relaxation of certain groups of muscles and 5. those which are indifferent to contraction and relaxation of muscles. Furthermore, preliminary analysis with the computer indicates that there is a close cross co-relation in the activity of cells of the cortex and of the thalamus. Finally, bilateral representation of motor activity could be demonstrated by single cell discharges recorded from the cortex and thalamus.

Significance to Bio-Medical Research: This investigation may lead to a new field in which the integration function of the human brain at a cellular level could be studied in patients subjected to brain surgery.

Proposed Course of Project: It is proposed that this study be extended to the cerebellum which is known to be of great importance in motor function.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Li, C-L., and Tew, J. M.: Reciprocal activation and inhibition of cortical neurones and voluntary movements in man: cortical cell activity and muscle movement. Nature, 203: 264-265, July 1964.

Li, C-L., Friauf, W., Cohen, G., and Tew, J. M.: A method of recording single cell discharges in the cerebral cortex of man. E. E. G. and Clin. Neurophysiol., 18: 187-190, Feb. 1965.

Honors and Awards relating to this project:

Serial No. NDB(I)-57 SN/CP 401(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Psychological Evaluation of Temporal Lobe Operations

Principal Investigator: Herbert Lansdell, Ph.D.

Other Investigators: M. Baldwin, M.D., A. Ommaya, F.R.C.S., C-l. Li, M.D., Ph.D., N. Urbach, M.A., P. Fedio, Ph.D., and J. Baker

Cooperating Units: None

Man Years
Total: 1.4
Professional: 0.9
Other: 0.5

Project Description:

Objective: To study neurosurgical patients with temporal lobe disorders with respect to intellectual abilities, visual and auditory perception, linguistic functions and other "personality" features.

Methods Employed: Intelligence and personality tests; aphasia, audiometric, and other specialized verbal tests; tests of visual perception.

Major Findings: In the light of our research indicating sex differences in the lateralization of some cerebral functions, some of the literature on anatomical data have been scrutinized. An interesting peculiarity was noted in Conel's data on the cortical area for hand representation in 5 yr. old children; the hemispheric differences in boys seemed to be opposite from that in girls. The small number of cases did not allow a satisfactory statistical conclusion; however, the literature on tactile thresholds and other considerations encouraged the speculation that the sex difference is a real one.

Our previous results showing sex differences in one-year-plus followup scores of temporal-lobe removal cases on the MMPI (a "personality" questionnaire) have been related to similar differences in the scores on another test, Graves Design Judgment Test (DJT). Previously, this design preference test had demonstrated sex differences in pre- vs. post-operative comparisons, but not a year or more after operation. The scores on this preference test have now distinguished among the followup groups in the differences in the correlations of the scores with the size of the removals. A similar pattern of correlations existed in the two tests, MMPI and DJT, in that the correlations in the male group with the right-temporal removals were of opposite sign from the other three groups. There was also a significant correlation between the DJT scores and the measure derived from the MMPI.

The interpretation of the results is at present limited by three considerations: a) some of the followup data are not consistent with the corresponding pre- vs. postoperative data, b) the relations to size of removal may reflect extent of original neuropathology rather than the extent of ablation, and c) the tests were not designed for neuropsychological research. The first limitation is an interesting challenge suggesting analyses of other test scores and other data on the patients; and as more patients are tested for the third time some understanding of the long-term postoperative changes may be obtained. The second limitation may also be subject to analysis as more patients are tested three times (most analyses, at present, involve different groups without adequate overlap in the selected cases). The last problem is exemplified by our results with the MMPI: as the most widely-used personality questionnaire it would seem to be an appropriate research instrument; however, the index which proved useful in our work was a "configural" method of scoring designed to aid psychologists in making differential psychiatric diagnoses. At present we have no satisfactory description of the meaning of this configural index in our setting; considerable caution seems warranted, since the delineation of personality factors as lateralized cerebral functions, particularly with sex differences, is likely to be more controversial than the old problem of lateralization of intellectual factors.

Significance to Neuropsychological Research: With careful selection of patients, relations between psychological test scores and extent of cerebral lesion can be established selectively for different loci and for each sex; these

relationships have implications for physiological concepts of "higher mental" processes.

Proposed Course of Project: Continued testing of patients undergoing surgery; further analysis and reporting of present results.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Lansdell, H., & Mirsky, A. F. Attention in focal and centrencephalic epilepsy. Exptl. Neurol., 9: 463-469, June 1964.

Lansdell, H. Sex differences in hemispheric asymmetries of the human brain. Nature, 203: 550, August 1st, 1964.

Serial No. NDB(I)-63 SN/CP 1032(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Psychological Effects of Subcortical Lesions Used for Relief from Abnormal Movements.

Principal Investigator: Herbert Lansdell, Ph.D.

Other Investigators: J. Van Buren, M.D., A. Ommaya, F.R.C.S., C-l. Li, M.D., Ph.D., P. Fedio, Ph.D., N. Urbach, M.A., J. Baker.

Cooperating Units: None

Man Years
Total: 0.7
Professional: 0.4
Other: 0.3

Project Description:

Objective: In comparison with the effects of other forms of neurosurgery, to investigate the impairments resulting from subcortical lesions with the aim of delineating subcortical from cortical factors in behavior.

Methods Employed: A battery of tests (the same as those used in Project Serial No. 67c) is given to patients before, two weeks after, and again a year or more after operation.

Proposed Course of Project: When a sufficiently large sample of cases has been tested, the data will be analyzed.

Part B included: No

Serial No. NDB(I)-63 SN/CP 1033(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Phonemic Aspects of Dysnomia

Principal Investigators: Herbert Lansdell, Ph.D.
Paul Fedio, Ph.D.

Other Investigators: J. Lansdell, J. Baker

Cooperating Unit: Rehabilitation Department, CC

Man Years
Total: 1.3
Professional: 1.1
Other: 0.2

Project Description:

Objective: To investigate the phonemic aspects of induced dysphasia and normal naming errors.

Methods Employed: Filmstrips and a projector are used. The filmstrips contain three orders of two sets of pictures; the names of the pictured objects have a wide range of phonemes in the initial and final positions. The nouns of one set of objects are of high frequency in the language; the other set includes, in addition, objects with medium-frequency names. The projector has an attached miniature screen and provides automatic control of exposure time from 4.4 to less than 0.5 sec/frame.

Major Findings: The basis of the present research is a previous study in which we found that the type of initial and final phonemes in the nouns influenced the number of dysnomic errors made by epileptic patients during the carotid Amytal test. Two recent experiments are of interest; one investigated the number of errors made by normal people when they attempted to name pictures presented too quickly for flawless performance; the other involved the errors made by patients whose brains are stimulated through implanted electrodes. Future work is contemplated on patients during neurosurgery and during the carotid Amytal testing.

Thirty subjects were tested twice in the naming of 72 pictures of objects projected rapidly on the small screen. Each subject was first leisurely trained to give the required names. Three filmstrips with different orders were used. The exposure rate was one frame every 0.45 sec, with alternate frames a picture of the word "and." The "and" pictures were used to help eliminate extended periods of silence by reactivating the vocal system after subjects omitted naming or incorrectly named the picture of an object.

The expected relation between phonemic incidence in the nouns and the errors in naming the objects was not found; however two other simple phonemic features proved to be relevant.

The greater the number of sounds, or phonemes, in the word the greater were the number of errors in the object-naming task; the number of syllables was not significantly related to the errors. Slightly more than half of the mistakes were substitution errors, and the initial phonemes of these substituted nouns were of higher frequency in the English language than those of the correct nouns.

Word frequency for the correct nouns was also a relevant factor in that objects with more common names produced fewer errors than objects with rarer names. However, the substituted nouns were not of higher frequency than the correct nouns. The individual level of errors bore no relation to ability on a vocabulary test.

Twenty-four college students were asked to rate the objects for familiarity (by use of a word list) and these ratings were found to be related to word frequency in the language but not to the errors obtained in the object-naming task.

None of the aspects of the errors found with the speeded task with normal people were relevant to the errors observed originally during the carotid Amytal tests on epileptic patients. Various differences between our conditions for obtaining Amytal-induced dysnomia and errors resulting from speed stress may be important, e.g., real objects rather than transilluminated pictures, epileptics rather than bright normals and so on. We would hope that a drug effect compared with speed disruption is the essential difference between the test situations. One of our original speculations was that the Amytal may selectively interfere with some inhibitory factor needed for correct selection among those initial sounds which are most often used in English. We might continue to

speculate that during fast vocal activity, substitution errors result from the strong habit strength of familiar sounds. In other words, Amytal may disrupt some auxiliary component of a system in the hemisphere for speech, while speed may overload some other component.

We conclude the original result has more limited implications than we had hoped for; the filmstrips will be used on patients in situations identical or similar to the original research to delineate further the various aspects of dysnomia.

A pilot experiment was undertaken to test patients during stimulation through intracranial electrodes situated in medial temporal and thalamic structures. An abbreviated form of the object-naming task was employed during the stimulation; a motoric and delayed recall feature was incorporated into the performance. In this form, the task permits an analysis of arrest errors, and those related to dysnomia, post-distraction aphasia and/or memory impairment. There seems to be a possibility of differentiating cortical and subcortical loci with different types of dysphasic errors. On the basis of the preliminary data, systematic investigation to evaluate various cognitive processes will be continued.

Proposed Course of Project: The apparatus will continue to be used in an attempt to confirm and extend the generality of our previous finding that the phonemic structure of the relevant nouns is related to various forms of dysnomic difficulties.

Part B included: Yes

Serial No. NDB(I)-63 SN/CP 1033(c)

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Lansdell, J. The carotid Amytal test. Md. J. Speech & Hearing, 8: 9-10, Sept. 1964.

ANNUAL REPORT
July 1, 1964 through June 30, 1965
Ophthalmology Branch, IR
National Institute of Neurological Diseases and Blindness

Ludwig von Sallmann, M.D.
Chief, Ophthalmology Branch

As in the past years, the investigations of the Ophthalmology Branch fall into the following main categories; physiology of vision, retinal disease, and the physiology and pathology of the uvea, intraocular pressure, and lens. All clinical projects had their counterpart in laboratory studies. Ramifications of the laboratory studies, in turn, often led to basic levels of research and were extended to work in other organ systems while remaining related to the main problem of visual dysfunction. In several instances detailed investigations of the ocular manifestations of rare systemic diseases, although not programmed, contributed to phenomenological and diagnostic aspects of the disorders. Certain studies on diseases of the cornea and ocular tumors were carried out but played a relatively minor part in the overall program.

From April 30, 1964 until May 1, 1965, 154 subjects (patients and normal controls) were admitted to the nursing unit for participation in the various projects. They accounted for 6,738 inpatient days. Data on the patient census are computed for the specified period since in the preceding report, figures from April to July 1964 were not included and the number of admissions for May and June 1965 cannot be anticipated. In the Outpatient Department 1,622 visits of 534 patients were listed in the records and 1,197 consultation requests were answered in this year. Major surgery was carried out on 26 patients and minor surgical intervention in 47 instances.

Active collaboration with other Institutes and Agencies was gratifying. The help of the following units is greatly appreciated: NCI, NIAMD, NIAID, NHI, and NIMH, Division of Biological Standards, Marine Biological Laboratory in Woods Hole, the A.R.C. Institute of Animal Physiology, Cambridge, England, and Fish and Wildlife Service of the U.S. Department of the Interior.

In the Visiting Scientist Program, Professor Antonio Borsellino from the Institute of Physics, University of Genoa and Dr. Rudolf Kern from the University Eye Department, Zurich, took part in the research activities, as did Dr. Fritz Baumann on an International Postgraduate Fellowship from Geneva, Switzerland.

Physiology of Vision

Praise is put forth for the effort and the considerable progress achieved by the Section on Physiology in obtaining basic facts necessary for understanding the mechanism of vision. New investigations on electrical interaction among different cells in a single ommatidium of the limulus paved the way for identifying the processes responsible for activating visual receptor cells. The relation between the light stimulus and electrical changes in

visual cells was shown to be characterized by two parameters with the dimensions of rates. One of these parameters controls the delay of responses to brief light flashes. Light adaptation reduces amplitude and speed of these responses but the rate of rise is not affected by the state of adaptation. In contrast to this disparity of response patterns, increase of temperature influenced speed and maximum voltage of responses to light flashes in the same proportion. Studies on temperature dependence of reaction rates suggests that a chemical reaction or reactions are operating in the production of visual responses. It is planned to possibly identify the chemical processes required for generation of visual responses. Also attempts will be made to characterize the sub-cellular structures which are primarily affected by light.

Other microelectrode studies on the limulus in which the light evoked potential changes were recorded simultaneously in the retinal and retinula cells, as well as in eccentric cells, indicated that all these cells are electrically connected. The retinula cells could be the only source of the generator potential and the electrical event may spread to the eccentric cells to initiate the nerve impulse.

Electrophysiological studies on the eye of the honey bee disclosed that the membrane of its visual cell has complex properties; some typical of a nerve membrane and others characteristic of a receptor cell membrane. The first type of property is deduced from the non-linear voltage-current relationship in these responses and the appearance of a fast transient. The second property may be responsible for the decrease of resistance induced by light adaptation. The shape of the responses to a light flash may be the expression of both of these properties.

In the Laboratory of Electroretinography, micro and macro electrodes were used to record electrical responses from the retina in vivo to analyze the light induced potentials in this tissue on a cellular level. Responses of various cells in the retina were separated by changing wave length and energy and by controlling the spatial distribution and time function of the light stimulus. A technique was developed to introduce under biomicroscopic observation fine glass micro electrodes in the surface layer of the intact eye of the anesthetized Rhesus monkey. The stimuli consisted of small spots or patterns of monochromatic light. This technique offered a significant opportunity to distinguish three functional types of ganglion cells in the central primate retina. These are, cells with spontaneous high frequency activity which was initially inhibited by light, cells with moderate spontaneous activity initially excited by light and, finally, cells which appear to code color information. The last group is excited by light at one end of the visible spectrum and inhibited by light originating at the other end. In the future, responses of single retina cells will be correlated with their histology. Optic nerves have been sectioned in order to isolate the responses of individual bipolar cells.

In the Laboratory of Cell Biology new physical-chemical studies are directed toward demonstrating the role which light-induced bleaching of rhodopsin plays in the excitation of the visual system. This area of study turned out to be productive and brought forth an array of fascinating facts

which cannot be summarized readily. Bleaching of rhodopsin in a monomolecular layer at an air water interface resulted in a slight decrease of surface tension. Retinene deposited underneath the rhodopsin monolayer exerted the same effect. The bleaching effect on the surface tension could be explained by penetration of retinene released from rhodopsin during bleaching into the rhodopsin layer.

Another aspect of the investigation was concerned with cation leakage of rhodopsin in artificial membranes, of rhodopsin micelles, and of isolated rod outer segments after illumination. Only the last method rendered positive results. It illustrated that in the dark there was slow outward leakage of both sodium and potassium from isolated rod outer segments of cattle. Following illumination there was a continuing loss of potassium but accompanied by an equal increase of sodium. Indeed, this effect could be brought about with weak light stimuli (2 seconds, 125 foot candles) which essentially caused no bleaching of rhodopsin. All-transretinene in low concentrations (10^{-6}) gave the same results. It is concluded that an ionic mechanism operates in the activation of the photoreceptors on illumination. Bleaching of just one molecule of rhodopsin causes a localized change of permeability of the rod sac resulting in Na-ion influx and K-ion outflow. The cation gradient might be reestablished via the sodium-potassium activated ATPase system. Theoretical calculations and directly observed data on cation movement were in good agreement with each other. It is proposed to strengthen the new theory of rod activation by further investigation.

Preliminary studies on glycoprotein synthesis in the retina have been initiated. Techniques will be utilized by which recently a single enzyme function in the terminal step of biosynthesis of the product was demonstrated. This enzyme is present in various tissues but predominantly in tissues with secretory function. It is probable that the retinal pigment epithelium belongs to these tissues and is active in transferring single sugars to incomplete glycoproteins. This reaction appears to be the final steps in the synthesis of the carbohydrate side chain of these glycoproteins. The glycoproteins in the vitreous humor may originate from glycoprotein synthesis on the retina. With the limited quantity of tissue material from the eye a continuing uphill struggle is anticipated in applying the necessary elaborate methods.

Another approach to solve retinal problems of biochemical nature is an attempt, in the Section of Chemistry, to obtain purified rhodopsin by extraction with a new detergent BC720, alkyl poly- (ethylene oxide) ethanol. When rod powder was treated with this detergent and analyzed in the analytical ultracentrifuge, the preparation exhibited two fairly sharp symmetrical peaks, one representing the Rhodopsin-BC720 complex and the other BC720 micelles. By lowering the concentration of the detergent the preparation approached a purity that resembled that arrived at by digitonin extraction. Chromatographic purification was easier on the BC720 rhodopsin material. Obviously it has to be shown first to which degree purification can be accomplished in order to steer clear of contaminations before difficult physical-chemical examinations are carried out.

Retinal Disease

Efforts expended in studies of degenerative diseases of the retina have been remarkably productive. New observations were numerous and trustworthy in view of the elaboration of methods of psychophysical and electrophysiological testing. More precise information became available on the functional disturbance in rare disease entities such as congenital stationary night blindness, fundus flavimaculatus, and acanthocytosis (a-beta-lipoproteinemia). The orderly investigation of the systemic disease, acanthocytosis, represents a first-rate cooperative effort which led to the major discovery that, in this form of tapeto-retinal degeneration, treatment with vitamin A in soybean oil improves retinal function. An achievement of practical significance was the refinement of static perimetry to permit recognition of loss of retinal function in certain retinopathies, such as that produced by chloroquine intoxication, at an early stage when other techniques fail to be of diagnostic value.

To this group of investigations on the retina belong experimental attempts to produce deterioration of the visual receptors by continued exposure of rats to light and by inducing accumulation of abnormal fatty acids by chronically feeding tetramethylhexadecanoic acid to rats. No results have been obtained so far in this study.

A multidisciplinary clinical approach in the study of vascular retinopathies has still not provided leads for the understanding of pathologic events resulting in severe visual loss. Neither routine laboratory testing nor hematologic, immunochemical and chemical (lipid), or histologic examinations of skin muscle biopsies turned up useful information as to the etiology of the disease.

Most recently workers in the Laboratory of Perinatal Physiology observed that young monkeys with experimental allergic encephalomyelitis (EAE) developed eye pathology. The histologic examination of eight eyes from this group of animals showed, in most instances, pathology of the retinal vessels or hemorrhagic conditions varying from a mild to a most destructive degree. The histopathologic involvement of the optic nerve was not related to the severity of the retinal lesions. These new findings are of interest since certain facts suggest that EAE is of allergic nature and that autoimmune mechanisms may be at work in some of the human vascular retinopathies accompanied by recurrent hemorrhages. It will be necessary to accumulate more material and to follow the developments of the ocular changes. If such material becomes available, it will be instructive to carry out electron microscopic work.

Uvea

Patients with uveitis of various age groups represent the greatest number of in-patients. Results of corticosteroid therapy and chemotherapy combined with hospitalization were satisfactory in a great number of the patients, but the etiology of the disease remained obscure as a rule, or was considered suggestive at best.

The use of Methotrexate (MTX) was continued in a selected group of patients with anterior uveitis. The most dramatic improvement was observed in a patient with sympathetic ophthalmia, whereas cases with cyclitis responded slowly but by and large favorably. Behcet's disease appeared refractory to the therapy. Immunologic studies on these patients supported the concept that MTX does not suppress delayed hypersensitivity and secondary antibody responses, in contrast to the inhibition of the primary antibody response and of the mononuclear phase of the inflammatory reaction as tested on the skin. The study will be extended to more patients in whom other forms of therapy have proven to be ineffective and to investigation of the advisability of long-term anti-metabolite treatment.

In an experimental study ischemia of the choroid was produced in the cat by injection of a thrombosing agent into a vortex vein. With this technique areas of the choroid vascular bed are eliminated whereas circulation in the retinal vessels is not altered. Thus the role of the choroid in nutrition of the retina can be studied under most favorable conditions. It could be demonstrated in histologic examination that the severe ischemia of the choroid produced severe damage of the photoreceptors and their nuclei and also of the pigment epithelium. Minimal ischemia caused injury solely to the cones and rods and the pigment epithelium. The experiments proved directly the dependence of nutrition of the outer layers of the retina on the choroidal circulation. Chronic experiments utilizing these new techniques are in progress.

Intraocular Pressure

In the last year the glaucoma project has been greatly expanded, both in its clinical as well as its laboratory phase. Aqueous humor dynamics and hemodynamics in diseased human eyes and in experimental animals were the subjects of several studies.

In a clinical investigation effects of topical administration of corticosteroids on aqueous outflow were re-examined by critically studying the influence of the steroids on the responses to a water load. Behavior of the normal and glaucomatous eye in the water drinking test, and effect of rapid changes in the arterial and venous pressure on the intraocular pressure were analyzed, and new methods were employed to study the interrelationship between autonomic nervous system activity and the intraocular pressure. An array of various techniques served in the different phases of these examinations.

The rise of the intraocular pressure in response to topically administered corticosteroids was rapid in familial open angle glaucoma, slower in secondary glaucoma, and delayed and slight in non-glaucomatous eyes. Responses to the water drinking test were definitely pathological when the steroid-induced pressure rise was large, but lowering of the C value was questionable in eyes with a slight response to steroid provocation. These findings are pertinent to the current controversy over the mode of action of corticosteroids on the outflow mechanism.

The relation of serum osmolarity to the rise of IOP in the water drinking test was thoroughly studied, and it was concluded that the osmotic effects are

not the only cause for the increase of IOP following ingestion of a large volume of water. It is not clear, however, which other mechanisms come into play in causing this pressure rise.

Different techniques were used to compare the responses of the systemic venous pressure and the IOP to increased intrathoracic pressure. Elevation of the intrathoracic pressure is followed by a slow rise of the venous pressure and a much more precipitous increase of the IOP. A certain independence of the two functions was demonstrated by varying the methods of inducing changes of the intrathoracic pressure. Glaucomatous eyes responded to this test in an irregular fashion.

Patients with glaucoma and normal controls did not exhibit any correlation of changes of the IOP and those of other autonomic functions such as skin resistance and pulse rate.

Throughout these studies it was obvious that the repeatability of tonographic measurements leaves much to be desired. Certainly this technique does not simply measure the mechanics of outflow. Its results are influenced by vascular, nervous, and possibly hormonal factors in addition to mechanical considerations. Future examinations may show whether the influence of these factors on the IOP can be defined so that they could be made the target of therapy.

The regulation of IOP by various factors particularly by reactions of the intraocular vasculature remained the main area of research in the Section on Pharmacology. Enucleated arterially perfused human and cat eyes were studied to delineate their usefulness as test objects for pharmacological and physiological studies. An ERG could be elicited from isolated eyes despite elimination of normal blood supply and nerve connections. The persistence *in vitro* of such a sensitive response increases the value of these preparations for pharmacological and physiological investigations.

C^{14}-labeled inulin was used to measure the aqueous humor turnover rate in cat eyes and this technique was applied in a series of experiments. It was established that in isolated eyes the rate of aqueous turnover was 35% lower and anterior chamber volume was 15% smaller than in eyes *in situ*. Re-investigation of the effect of acetazolamide with this method demonstrated that, in small doses, the drug lowers IOP without decreasing the rate of aqueous humor formation. Much higher doses are required to achieve the latter effect. These results are in agreement with the concept that, under the experimental conditions, acetazolamide decreases IOP by a vascular mechanism. The hypotensive response induced by ouabain was also examined, and in this case, a dose-dependent decrease of aqueous humor formation was observed.

In conjunction with these experiments, the effects of acetazolamide and epinephrine on vessels of the isolated lateral chorioplexus of the cat were investigated. The technique of perfusion of the chorioplexus was the same as that used in isolated iris preparations. Acetazolamide and nor-epinephrine caused vasoconstriction of the plexus vessels but not of the cerebral vessels, whereas eserine, ouabain, histamine and serotonin had a constrictor effect on both the plexus and brain vessels.

The effects of betamethazone, a compound which increases the IOP in man on chronic local application, were examined on the vasculature of arterially perfused irides. It appears to act as a smooth muscle depressant and prevents the vasoconstriction induced by eserine, ouabain, arterenol, and acetazolamide. A rise of IOP following betamethazone perfusion was also shown in the enucleated eyes.

In another study an attempt was made to explain the nature of the uniform vasoconstrictor response of the iris artery to sympathomimetic and parasympathomimetic agents as well as to histamine, ouabain, and acetazolamide. From the analysis of the results obtained with the use of blocking agents it was concluded that the parasympathomimetic agents act by stimulating cholinergic post-ganglionic receptors whereas the catecholamines appear to stimulate post-ganglionic sympathetic receptors. The action of ouabain and acetazolamide may be caused by direct stimulation of smooth muscle fibers. The strange similarity of the responses of the iris artery to pharmacological antagonists is not explained by the results of this study and require further investigation. Work on the corticosteroids has to be expanded in view of the great practical interest in the ocular effects of local steroid therapy.

A bioassay, previously designed for analysis of the effects of pharmacologic agents on muscle strips of iris and ciliary body, proved of great value in the study on the action of corticosteroids on these muscles. It was clearly demonstrated that betamethazone depresses alpha receptors of the sphincter and the dilator of the iris and of the ciliary body. Very low concentrations of the drug reduced greatly the effect of acetylcholine, l-epinephrine, l-norepinephrine or serotonin. The alpha receptors of the sphincter appeared to be more sensitive to the effects of low doses of betamethazone than those in the dilator. The negative myotropic effect in the strips of intraocular smooth muscles has significance in the interpretation of steroid induced intraocular pressure rises. Smooth muscles other than those of the eye will be tested for comparison.

Studies on the extraocular muscles represent a new area of investigation in this Branch. Again use was made of the techniques previously devised for intraocular muscle strips. It was discovered that in the superior rectus muscle of the rabbit slow and twitch fibers form individual muscle plates which can be separated from each other by careful dissection. Histologic examination indicated that there is practically no mixture of fibers in the two layers. The slow fiber preparation responded to acetylcholine with tetanic contractions whereas the twitch fibers which make up the main bulk of the muscle were either refractory or showed only transient and slight responses. The latter could be explained by the presence of a few slow fibers in the twitch fiber portion of that muscle. Experiments provided direct evidence that the slow fibers maintain the muscle tone. It is hoped that electrophysiological studies on single twitch and slow fibers can be conducted in the future.

Another phase of this study was concerned with the distribution and nature of adrenergic receptors in extraocular muscles in cat, monkey and rabbit. It was shown that the recti and oblique muscles of the rabbit and cat have alpha and beta receptors whereas in the corresponding muscle of the monkey only beta

type receptors were demonstrated. It is probable that the slow fibers in the superior rectus of the rabbit carry the adrenergic receptors. The study will be continued on muscle of other species.

Cornea

Infectious and degenerative diseases are being continuously investigated, particularly with regard to fungus infections, a project which was included in last year's report.

In the Section on Chemistry, corneal collagen was submitted to extensive physical-chemical studies. The molecular weight, the molecular dimensions, and the helical structure were similar to those of other collagens, but differences were noted in the amino acid composition and in the temperature range for helix to coil transition. A collagen has been isolated which not only appears to be monomeric, but has minimal intramolecular cross-linking. In contrast to the very narrow transition zone of the polymeric corneal collagen, the monomeric has a transition zone of a width characteristic of collagen of other sources. This implies that the structural stability of the polymeric form can be attributed to intramolecular links between the subunit chains and intermolecular cross-linking between monomeric collagens forming the polymer. These findings might be of functional significance. The data provides a base for comparative studies of different collagens of the eye and for studies of the role of collagen in corneal development, and in corneal pathologies. Studies of such material are in progress.

Lens

The activities of the Section on Cytology and Histopathology have been oriented primarily toward problems of cataractogenesis. In histological and histochemical studies on lenses obtained from the operating room no changes have been observed which would throw light on the mode of action of certain cataractogenic agents such as MER-29 or cortisone or on the significance of systemic diseases such as agammaglobulinemia or local diseases such as uveitis for the development of lens opacities.

An interesting new experimental cataract has been produced in young trout by long-term feeding of thioacetamide, a compound known to produce hepatomas in rats. Biomicroscopically, the opacity appears to originate at the anterior pole and progresses to engulf most of the lens cortex. Histological examination demonstrated a circumscribed proliferation of the lens epithelium at the anterior pole. The cells here seemed to invade the underlying cortical fibers. It, as yet, undetermined whether the active proliferation of epithelium is the initial event or whether this proliferation is simply a response to a cortical lesion. The question is of considerable interest in view of the carcinogenic nature of thioacetamide and the fact that cancer of the lens has never been observed. It is planned to produce this cataract in rats, a more convenient experimental animal, so that studies of the developing opacity and of induced cytologic changes can be more carefully controlled.

Studies on the normal population dynamics of the lens epithelium and of the influence of certain cytotoxic and cytostatic agents on the cell system have been continued. Investigations on the antimitotic action of the alkylating

agent, triethylene melamine was extended to an examination of the effects of the drug on DNA synthesis as determined by incorporation on H^3-thymidine and autoradiography. From the observations made in this prolonged study, it is concluded that triethylene melamine significantly reduced the rate of DNA synthesis and that its mechanism is markedly different from that of a previously studied alkylating agent, Myleran.

Investigations of the action of Myleran were continued in a study of the effect of chronic administration of the drug and, as expected from the postulated mechanism of action of the compound, profound depletion of the epithelial cell population was observed. This accounts for the known cataractogenic property of myleran in animals.

Continuing efforts to establish the pattern of normal population dynamics in this cell system as a prerequisite for studies of toxic agents led to experiments on the diurnal variations in mitosis and DNA synthesis, and the effect of age on these rythmns. Preliminary results demonstrated that the extent of moderate fluctuation of mitotic activity observed in young animals does not appear to change with age, although there appears to be a time shift of the periods of maximum and minimum activity. Corresponding to the variations in mitotic activity there is a marked fluctuation in the number of DNA synthesizing cells of nearly the same magnitude, but approximately 12 hours out of phase. This phase difference may be explained by the timing of events in the cell cycle in which the initiation of DNA synthesis precedes mitosis by about 12 hours.

Systematic work in the laboratory of electron microscopy had to be interrupted because the head of this unit has not yet been replaced. The facilities of the laboratory have been used in connection with histologic studies in a few instances but basic research had to be postponed until the position of the head of the laboratory is filled. However, during this time some technical improvements on the electron microscope have been accomplished.

Ocular Changes in Systemic Disorders

In this report year cooperation with investigators of other institutes was particularly fruitful in studies of systemic diseases in which involvement of the eye played an important part. Reference has been made to the discovery of the beneficial effect of Vitamin A treatment on the Tapeto-Retinal degeneration which accompanies acanthocytosis, a systemic disorder characterized by the absence of a beta-lipoprotein. New ophthalmologic observations were described in other disease entities. In fifteen patients with carcinoid disease, prodromal signs, such as severe lacrimation and injection of conjunctival vessels presaged the onset of a true flush. Retinal changes consisted either of multiple retinitic spots in the macular area or exudative lesions in the periphery, in addition to punched out choroidal atrophic foci. The latter may have developed from the exudative process. They are thought to be connected with vascular pathology developing during the characteristic attacks.

In six children with cystic fibrosis of the pancreas, various stages of optic nerve involvement were encountered, ranging from retrobulbar neuritis of moderate degree to total atrophy of the optic nerve. Histological examination demonstrated degeneration of the retinal nerve fiber layer and demyelination and loss of axon cylinders in the optic nerve. It is generally accepted that this nerve pathology is produced as a toxic side effect of prolonged chloramphenicol therapy.

15 members of a family, in which the Ehlers-Danlos Syndrome was inherited, exhibited the characteristic manifestations of the disorders. Ophthalmoscopic examination disclosed as a new finding the presence of angioid streaks in the propositus and her daughter without signs of pseudoxanthoma elasticum.

Finally, 39 members of two families in which Fabry's disease (a glycolipidosis) occurred were studied ophthalmologically. In addition to previously described ocular manifestations of this disease a characteristic delicate cataract was observed for the first time. Genetic studies support the concept of sex linked transmission.

Serial No. NDB(I)-60 O/OPS 700 (c)
1. Ophthalmology Branch
2. Section on Ophthalmology Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Activity of Visual Receptor Organs

Principal Investigator: M.G.F. Fuortes, M.D.

Other Investigators: Fritz Baumann, M.D.
Antonio Borsellino, Ph.D.
Rosalind B. Marimont, B.A.
Thomas G. Smith, M.D.

Cooperating Units: Marine Biological Laboratory
Woods Hole, Massachusetts

Institute of Physics
University of Genoa, Italy

Laboratory of Neurobiology, NIMH

Laboratory of Neurophysiology, NINDB

Man Years (computed for the 12 month period)

Total:	2.58
Professional:	2.58
Other:	0.0

Project Description:

Objectives: The aim of the present research is to identify the processes responsible for activation of visual receptor cells.

Methods Employed: The methods employed are essentially the same as described in previous years. Electrical changes evoked by illumination are recorded from photoreceptor cells or visual nerve cells in eyes of different animals. The experimental results are compared to theoretical curves. In addition, studies have been performed with the use of two microelectrodes implanted simultaneously in two different cells of the same ommatidium, and other work has involved the study of the effect of temperature on visual responses.

Major Findings: A theory developed in previous work performed in collaboration with Dr. A.L. Hodgkin of Cambridge, England defines the relations between light applied to the eye and electrical changes occurring in visual cells. This relation is characterized by two parameters with the dimensions of rates. One of these parameters controls the rate of decay of responses to brief flashes. It was observed that responses to a given flash of light became smaller and faster when the visual cells were light adapted; these and other results could be interpreted assuming that light increases the rate of decay of visual responses without affecting the rate of rise. However, since temperature influences the rate of many physical and chemical processes, it was thought that changes of temperature should alter both the rate of decay and the rate of rise of visual responses. Thus, the theory proposed by Dr. Hodgkin predicts that the changes produced by temperature should be quite different from those produced by light adaptation. It was found that increase of temperature increased both speed and maximum voltage of responses to flashes of light. These effects are almost precisely what would be predicted by the theory if temperature changes both rate of rise and rate of decay in the same proportion. The possibility was then considered that the processes involved in the production of visual responses might be a chemical reaction. If this were the case, a definite relation should be found between reaction rates and temperature (Arrhenius relation). It was found that this relation is satisfied with reasonable accuracy in the experimental results.

Finally, it was noted that (according to the theory), if rate of rise and rate of decay are both changed in the same manner by temperature, the steady-state responses should not change when temperature is changed. This prediction was essentially confirmed by the results.

It may be justifiable to conclude from this study that the theory proposed in previous years provides an adequate and simple interpretation of the effects of temperature on visual responses and that the results are consistent with the hypothesis that a series of chemical reactions is involved in the production of visual responses.

Work on visual receptors of the honeybee has been continued by Dr. Fritz Baumann, a guest worker from the University of Geneva, Switzerland. In addition, a series of experiments has been performed in collaboration with Dr. Thomas G. Smith on the eye of Limulus during a stay in Woods Hole, Massachusetts.

The main results are as follows:
Honeybee: The response of a single visual cell to a flash of light consists of a slow depolarization of the cell membrane. When the depolarization reaches a critical value a fast transient depolarization followed by a positive after-potential appears superimposed on the slow wave. This fast transient has certain properties of a nerve spike: in certain limits it obeys the law of all-or-nothing; it can be triggered by depolarizing currents; and it is abolished by Tetrodotoxine,

a substance which presumably blocks sodium activation. The fact that the transient is not abolished by a separation of the visual receptor cell from its axon indicates that it originates in the membrane of the visual cell.

Some electrical properties of the visual cell membrane have been studied by measuring the potential changes evoked by hyperpolarizing and depolarizing currents applied through the intracellular microelectrode. When voltage is plotted against current, a curve closely resembling those obtained by Yamasahi and Nasahashi on an insect axon is obtained. The relation between voltage and current is not linear as found in other receptor cells. In the visual cell of the bee, as in the axon of the cockroach, the resistance of the membrane, determined by the slope of the voltage-current curve, increases when the cell is hyperpolarized; it decreases when it is depolarized. In addition, by comparing the slope of the voltage-current curve determined in dark- and light-adapted eyes, it was found that light decreases the membrane resistance.

From these results, it is concluded that the membrane of the visual cell of the bee has complex properties--some typical of a nerve membrane (responsible for the non-linear voltage-current relation and the fast transient) and some typical of a receptor cell membrane (decrease of resistance by the adequate stimulus). The shape of the response to a flash is probably determined by both these properties.

Limulus: By passing current through two microelectrodes inserted in the same ommatidium and recording the evoked potential changes in both cells, it was found that the different retinal cells and retinula cells and eccentric cells are electrically connected. It is possible that the generator potential originates only in one type of cell, the retinula cell and spreads electrotonically to the eccentric cell where nerve impulses are initiated.

Significance to Program of Institute: It is hoped that these studies on activity of visual receptors will lead to a better understanding of the operations responsible for visual responses and that in time they may lead to the identification of the mechanisms involved in these processes. Such knowledge would be important not only for a better understanding of problems dealing with pathology and physiology of vision, but also for a general interpretation of receptor activities.

Proposed Course of Project: An attempt will be made to identify what chemical processes may be required for generation of visual responses. Other studies will be performed in an attempt to identify the subcellular structures which are primarily effected by light. Comparison of results obtained in different animal species will probably be important for reaching these aims.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Fuortes, M. G. F. and Hodgkin, A.L.: Changes in Time Scale and Sensitivity in the Ommatidia of Limulus. J. Physiol. (London) 172:239-263, 1964.

Smith, T. G., Baumann, F., and Fuortes, M. G. F.: Electrical Connections between Visual Cells in the Ommatidium of Limulus. Science 147:1446-1448, 1965.

Honors and Awards relating to this project: None

Serial No. NDB(I)-63 O/OPS 1016(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Electrophysiological Studies of Mammalian Retina

Principal Investigator: Peter Gouras, M.D.

Other Investigators: Ronald E. Carr, M.D.
Ralph D. Gunkel, O.D.

Cooperating Units: None

Man Years (computed for the 12 month period)

Total:	1.6
Professional:	1.0
Other:	0.6

Project Description:

Objectives: To determine the cells responsible for generating the electrical activity of the retina following light stimulation and to understand their coding of visual information.

Methods Employed: Micro- and macro-electrodes are introduced into the eyes of animals in order to determine the sources of the light induced potentials of the retina. The responses of different retinal elements are separated either by suitable control of the wavelength, energy, spatial distribution and time function of the light stimulus or by the production of retinal degeneration. Retinal degeneration is later studied by light microscopy. Recently it has become possible to introduce fine glass micro-

electrodes to the retinal surface of the intact eye of the anesthetized Rhesus monkey. The responses of single cells are recorded to spots and patterns of monochromatic light. Both the microelectrode and the light stimuli can be clearly observed on the retinal surface. Cells in the foveal and central areas of the retina, in particular, are studied. The eyes can be restudied repeatedly over many months.

Major Findings: Three distinct functional types of ganglion cells have been found so far in the central primate retina: 1) Cells whose high frequency spontaneous activity is initially inhibited by light, 2) Cells with little spontaneous activity which are initially excited by light. Both of these cells receive information from both rod and cone receptors. 3) Cells which are excited at one end and inhibited at the other end of the visible spectrum. The latter cells appear to mediate an opponent mechanism of color vision in the primate retina.

Significance to Program of Institute: Such animal experimentation provides a means of correlating function with anatomy at the cellular level in a way that is impossible with the human retina.

Proposed Course of Project: To continue these studies of the monkey retina in an attempt to correlate function with the histology of single cells. In particular the optic nerve has been sectioned in Rhesus monkeys in an attempt to isolate the responses of single bipolar cells.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Gouras, P., and Carr, R.E.: Light-induced d.c. responses of monkey retina before and after central retinal artery interruption. Invest. Ophthal. (in press), 1965.

Gouras, P., and Carr, R.E.: Cone activity in the light-induced d.c. response of monkey retina. Invest. Ophthal. (in press), 1965.

Gouras, P.: Saturation of the Rods in Rhesus Monkey. J. Opt. Soc. Amer. 55: 86-91, Jan. 1965.

Gouras, P.: Primate Retina: Duplex function of dark adapted ganglion cells. Science 147: 1593-1594, Mar., 1965.

Honors and Awards relating to this project: None

Serial No. NDB(I)-65 O/CB 1209(c)
1. Ophthalmology Branch
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Mode of Action of Rhodopsin

Principal Investigator: Sjoerd L. Bonting, Ph.D.

Other Investigators: Alex Bangham, M.D., Department of Physiology

Cooperating Unit: A.R.C. Institute of Animal Physiology, Cambridge, England

Man Years (computed for the 12 month period)

Total:	1.0
Professional:	0.9
Other:	0.1

Project Description:

Objectives: To determine how the bleaching of rhodopsin by light in the mammalian rod receptor leads to excitation of the visual system.

Methods Employed:

(1) Physical studies of rhodopsin spread in a monomolecular layer at an air-water interface. Effects of bleaching of rhodopsin on surface tension and phase boundary potential were determined. Penetration of all-trans retinene in the rhodopsin monolayer was studied.

(2) Cation leakage studies with rhodopsin in artificial membranes systems, with rhodopsin micelles and with isolated rod outer segments after illumination.

Major Findings:

(1) Bleaching of rhodopsin in monolayer led to a small decrease in surface tension, interpreted as an expansion of the layer. Retinene, placed underneath a rhodopsin monolayer, penetrated into the layer, also leading to a decrease in surface tension. Qualitatively and quantitatively the effects of retinene were such that the bleaching effect on the surface tension may possibly be explained by penetration of retinene, released from rhodopsin during bleaching, into the rhodopsin monolayer.

(2) Results of cation leakage studied with rhodopsin in artificial membranes and with rhodopsin micelles were negative, presumably due to technical difficulties. Isolated rod outer segments from cattle retina, suspended in isotonic sucrose, showed a constant loss of potassium and an equal gain of sodium upon illumination, superimposed on an outward leakage of both ions in darkness. Even two seconds of illumination at 125 ft. candles, which did not bleach a detectable amount of rhodopsin, caused this effect. All-trans retinene, in concentrations from 10^{-4}M to as low as 10^{-6}M (1 molecule retinene per 100 molecule rhodopsin), caused the same effect.

Significance to Program of Institute: The constant ionic effects of light over a 5000-fold range down to amounts, too small to cause detectable bleaching of rhodopsin and of retinene over a 100-fold concentration range down to a molar ratio of retinene to rhodopsin of 1:100 suggest an ionic mechanism for the activation of the photoreceptor cell upon illumination. Bleaching of as little as one molecule of rhodopsin would cause a localized change in cation permeability of the rod sac membrane, heading to a sudden inflow of Na ions and an outflow of K ions just as in a stimulated nerve. The resulting depolarizing current would stimulate the bipolar cell via the receptor-bipolar cell synapse. The cation gradient would be reestablished by the Na-K activated ATPase system, previously shown to be present in high activity in the outer segment. Theoretical calculations show the feasibility of this mechanism in terms of number of ions moved, fraction of ions present, surface area involved, and cation pump capacity needed for recovery of the cation gradient. There is agreement between the calculated and observed cation movements. The cation pump capacity becomes limiting at the light levels, where rod saturation begins to occur.

Proposed Course of Project: This project will be continued in an effort to strengthen the evidence for the proposed theory of rod activation.

Part B included No

Serial No. NDB(I)-65 O/OCH 1210(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Chemistry of Rhodopsin

Principal Investigator: Marc S. Lewis, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years: (Computed for the 12 month period)
Total: 0.3
Professional: 0.1
Other: 0.2

Project Description:

Objectives: To seek improved methods of extracting and purifying rhodopsin in order to permit more meaningful studies of the physical and chemical properties of this lipo-protein.

Methods Employed: Rod powder was prepared from bovine retinas by conventional techniques and extracted with a variety of non-ionic detergents. The detergent solutions and the extracted solutions were studied in the analytical ultracentrifuge and spectrophotometrically.

Major Findings: It was found that in order to extract rhodopsin it was necessary to use a micelle forming detergent at a concentration above critical micelle concentration. Of the detergents tried, an alkypoly-(ethylene oxide) ethanol, Emulphogene BC-720 (Antara Chemicals) was found the most effective. When rod powder was extracted with a 1% solution of BC-720, the resultant solution exhibited two fairly sharp symmetrical peaks in the analytical ultracentrifuge. One of these was identified as the rhodopsin-BC-720 complex, the other, with approximately half the sedimentation rate, as BC-720 micelles. If the BC-720 concentration was lowered to where the peak associated with the BC-720 micelles disappeared, the rhodopsin-BC-720 peak broadened considerably, probably indicating variable BC-720 content in the rhodopsin-BC-720 complex. The results of these studies indicate that it is possible to obtain rhodopsin extracts with BC-720 which are comparable in purity to those obtained with digitonin. BC-720 appears to have an advantage over digitonin in terms of its behavior on chromatography columns.

Significance to Program of Institute: This study indicates that it is now possible to obtain a rhodopsin-detergent complex in a form more suitable for purification than has been possible with rhodopsin-digitonin complexes. It increases the probability of being able to do significant structural studies on rhodopsin.

Proposed Course of Project: It is planned to attempt further purification of rhodopsin by column chromatography, and, if the purity of the product obtained warrants, pursue studies involving determination of molecular weight, dimensions, amino acid composition, and possibly amino acid sequence.

Part B included: No

Serial No. NDB(I)-65 O/CB 1211(c)
1. Ophthalmology Branch
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Studies on Glycoprotein Synthesis

Principal Investigator: Paul J. O'Brien, Ph.D.

Other Investigators: Elizabeth F. Neufeld, Ph.D.

Cooperating Unit: Laboratory of Biochemistry and Metabolsim, NIAMD

Man Years (computed for the 12 month period)
Total: 1.8
Professional: 1.0
Other: 0.8

Project Description:

Objectives: To determine how the sugar residues of glycoproteins are added to the polypeptide core. To study the enzymatic reactions involved and the control mechanisms that regulate these reactions. To determine how altered, incomplete or defective glycoproteins might result from a disturbance of these reactions.

Methods Employed: The microsomal fraction of several rat tissues is isolated. The transfer of radioactive sugars from "activated" sugar nucleotides to endogenous acceptors is measured. The labeled products are liberated from the microsomes and identified by electrophoresis and immuno-chemistry. Transfer to heterologous acceptors is also measured. The effect of protein synthesis inhibitors is also studied.

Major Findings:

(1) Several tissues, including liver, kidney, small intestine, mammary gland and eye (probably the retina) are capable of transferring N-acetyl neuraminic acid (NAN) from CMPNAN to an unfinished glycoprotein bound to the microsomes. This transfer reaction probably represents the final step in the synthesis of the carbohydrate side chains of these glycoproteins since NAN is the terminal sugar of many glycoproteins.

(2) The products of this reaction in the rat liver microsomal system are tentatively identified as plasma glycoproteins on the basis of their electrophoretic properties.

(3) Puromycin, an inhibitor of protein synthesis, reduces the level of endogenous acceptors as much as 90%.

(4) The transfer of NAN to heterologous acceptors is apparent in all tissues studied. This implies that the transferase is not specific for proteins made by its own cell type. Rather, the specificity lies in the protein synthesizing system of the cell. The presence of this enzyme activity in any given tissue can be taken as presumptive evidence of the ability of that tissue to make its own glycoproteins.

Proposed Course of Project: To examine the nature of the product of this reaction in retinal tissue in an attempt to determine whether this tissue is responsible for the synthesis of glycoproteins found in the vitreous humor. To study the mechanism of addition of the other non-terminal sugars of glycoproteins. Synthesis of glycoprotein by other tissues of the eye will be studied.

Part B included No

Serial No. NDB(I)-63 O/OC 1012(c)
1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Electrophysiologic and Psychophysical Studies of Tapeto-Retinal Degenerations - Clinical and Experimental

Principal Investigator: Ronald E. Carr, M.D.

Other Investigators: Peter Gouras, M.D.
Ralph D. Gunkel, O.D.
J. Brooks Crawford, M.D.

Cooperating Units: Gastroenterology Unit, NIAMD
Laboratory of Metabolism, NHI

Man Years (computed for the 12 month period)
Total: 1.7
Professional: 1.3
Other: .4

Project Description:

Objectives: To study by the use of electrophysiologic and psychophysical techniques specific characteristics of the abnormal visual system in man and animals.

Methods Employed:

(1) Electrophysiologic - the electroretinogram, electro-oculogram, and electroencephalogram are used to study the light induced potential, the corneo-retinal potential, and the occipital evoked response.

(2) Psychophysical - by the use of dark adaptation, retinal profiles to monochromatic light, static perimetry, and color testing, the subjective response of the patient can be tested.

(3) The use of a digital computer so that responses of 1 uV of light or less are readily detected.

Patient Material: Patients have been obtained from both the outpatient and ward services of the Ophthalmology Branch and also referred

from the Institute of Arthritis and Metabolic Diseases of NIH. Normal subjects were obtained from the Normal Control Program of NIH.

Major Findings:

(1) Patients with congenital stationary night blindness without fundus changes have been further investigated. Genetic studies have confirmed the existence of both dominant and recessive forms and specific ERG patterns in each type.

(2) Patients with the disorder of a-beta-lipoproteinemia, a neurologic disorder associated with retinitis pigmentosa, have now been followed for a period of two years. These patients show a definite electrophysiologic and psychophysical response to Vitamin A therapy. Since the metabolic abnormality is one which leads to a generalized disorder of lipid metabolism, the exact pathogenesis of the retinal disorder cannot be elucidated. Various other factors which might play a role in this disease are now under investigation, but the fact that Vitamin A in soybean oil therapeutically is of benefit in a tapeto-retinal degeneration is the first observation made in humans that there is a relationship between this vitamin and retinal degeneration.

(3) Patients on varying dosages of chloroquine were investigated using perimetric retinal thresholds to red light in addition to other psychophysical and electrophysiologic tests. The range of reliability of this test was determined in a group of normal subjects and compared with those on chloroquine. It was found that this test was the most sensitive method to date for determining early retinal dysfunction due to chloroquine. A dose-sensitivity relationship between total dose of chloroquine taken and the retinal threshold was found, and a statistically significant elevation of threshold occured when the total dose of chloroquine exceeded 100 grams. Patients on chloroquine are particularly well-suited for this test since the foveal area is left intact and fixation can be maintained. Also, the earliest signs of chloroquine retinopathy occur in the macular and paramacular area, the region of greatest sensitivity and least variability of this test.

(4) A recent biochemical study, that of a high accumulation of an abnormal fatty acid in the blood of patients with Refsum's disease, has led to an experimental study of the effect of this fatty acid in rats. In conjunction with the NHI, rats are being fed 3,7,11,15 tetramethylhexadecanoic acid and serial electroretinograms and histologic studies are being performed. At the present stage no histologic or ERG abnormalities have been noted.

(5) The effect of prolonged, high-intensity light as a toxin to the retina is being investigated. In the past it has been shown that certain nocturnal animals, when exposed to light for a long period, will develop

a tapeto-retinal degeneration. It has further been shown that under nocturnal conditions, animals with inherited retinal dystrophy will develop this dystrophy more slowly then those in a normally-lighted situation.

New-born rats are being exposed to a constant illumination and serial electrophysiologic and histologic studies will be done in an attempt to produce a tapeto-retinal degeneration.

Significance to Program of Institute: The mechanism underlying these ocular disorders is being investigated and may contribute to a better understanding of their pathogenesis.

Proposed Course of Project: Clinical patients with the above noted and allied disorders will be investigated. Animal experimentation will continue on the above-noted projects.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Gouras, P., Armington, J C., Kropfl, W., and Gunkel, R. D.: Electronic computation of human retinal and brain responses to light stimulation. Ann. N.Y. Acad. Science 115: 763-775, July 1964.

2. Gouras, P., and Carr, R. E.: Electrophysiologic studies in early retinitis pigmentosa. Arch. Ophthal. 72: 104-110, July 1964.

3. Carr, R. E.: Central areolar choroidal dystrophy. Arch. Ophthal. 73: 32-35, Jan. 1965

4. Carr, R. E., and Gouras, P.: Oguchi's disease. Arch. Ophthal. (in press)

5. Carr, R. E.: Fundus flavimaculatus. Arch. Ophthal. (in press).

Honors and Awards relating to this project: None

Serial No. NDB(I)-65 O/CH 1212(c)
1. Ophthalmology Branch
2. Section on Cytology and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Ocular Pathology in Experimental "Allergic" Encephalomyelitis in Young Monkeys

Principal Investigator: Ludwig von Sallmann, M.D.

Other Investigators: Edwin M. Lerner II, M.D.
Ronald Myers, M.D.

Cooperating Units: Laboratory of Germfree Animal Research, NIAID
Laboratory of Perinatal Physiology, NINDB

Man Years (computed for the 12 month period)

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: This study is a part of a larger investigation of experimental "allergic" encephalomyelitis produced in baby monkeys by sub-cutaneous injection of guinea pig spinal cord. Eye pathology will be evaluated and correlated with brain pathology.

Methods Employed: The eyes of experimental animals are prepared for histological examination using several specific staining techniques in addition to routine procedures. At present 8 eyes have been examined. They will be more critically evaluated when the eyes of control animals have been prepared for comparison.

Major Findings: This is the first observation of extensive histopathological changes in the eyes of monkeys with experimental "allergic" encephalomyelitis. The changes involve the retinal vasculature predominantly; they may be limited to a few small hemorrhages with the destruction of adjacent tissues in various layers of the retina or may be so massive that almost no part of the retina escapes destruction. Diffuse hemorrhagic infarction of the inner layers are combined with hemorrhages in other parts of the retina which may extend into the retroretinal and vitreous spaces. The retinal vessels are engorged; the veins show all stages of thrombosis; and the arteries are partially occluded by endothelial proliferation and hyalinization. The optic nerve can be free of inflammatory changes or show extensive destruction with chronic perivascular cuffing as described by previous investigators. In rare instances, the uvea shows inflammatory changes.

Significance to Program of Institute: Vascular retinopathy is one of the projects of the clinical unit of the Ophthalmology Branch. As a rule the search for the cause of the blinding disease proves futile despite the cooperation of various laboratories and medical consultation. The possibility that the autoimmune process plays a part in the recurrent hemorrhages deserves consideration although up to the present no proof for such a mechanism is at hand. If experimental "allergic" encephalomyelitis is considered to be of allergic nature the observation of a destructive hemorrhagic retinitis in the young monkeys is of special interest.

Proposed Course of the Project: It is planned to make available to Drs. Lerner and R. Meyer all of the findings of ocular pathology observed in their group of animals for the purpose of comparing the histology of the lesions in the central nervous system and those in the eye. When more animals become available other stages of retinopathy and optic nerve disease can be followed systematically.

Part B included: No

Serial No. NDB(I)-63 O/OC 1022(c)
1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Methotrexate Therapy of Selected Patients with Uveitus

Principal Investigator: Vernon G. Wong, M.D.

Other Investigators: Evan Hersh, M.D.

Cooperating Branch Medicine Branch, NCI

Man Years: (computed for the 12 month period)
Total: 0.5
Professional: 0.5
Other: 0.0

Project Description:

Objectives: This is the continued project initiated January, 1963 to study the therapeutic effect of methotrexate on selected and refractory cases of uveitis. Inflammatory and immune responses were simultaneously studied in these patients during treatment.

Methods Employed: (1) Twenty-five mgms. of Methotrexate were given one week prior to the start of treatment to rule out any idiosyncratic reactions. Methotrexate was then injected intravenously, at a dose of 25 mgms./M^2 body surface area every four days for six weeks. Complete blood counts and blood chemistries were obtained prior to each injection of the drug to rule out any toxic side effects.

(2) The local inflammatory response was studied by the skin window technique. The midvolar surface of the forearm was abraded with a No. 10 Bard-Parker blade to an area of 1 cm^2. The abrasion was carried to the papillary layer of the corium as evidenced by pin-point bleeding. The area was then covered by a cover slip which was held secure by adhesive. Changes of cover slips were done at 4, 8, 24, and 48 hours. The cellular exudate adherent to the cover slip was stained with Wright's stain. Two hundred or 400 cell differential counts were done on each preparation. The 400 cell differential counts were done when the exudate was not of uniform cell type.

(3) The ability of these patients to form antibodies during therapy with Methotrexate was determined by challenging them with a known foreign

antigen one week after the start of therapy. Blood was obtained at weekly intervals until cessation of therapy for determination of antibody titers Any existing titer of antibody was ruled out by pre-treatment examination of the patient's serum. The antigens used were cell wall extracts from E. coli (V_1), Tularensis (Tul), and Pneumonococcus Type III (Pn). The dose of each was 100 mcg/ml.

The antigens used in this study were obtained from the Medicine Branch of the National Cancer Institute. Determination of antibody titers in these patients was performed by the National Cancer Institute.

Major Findings: To date 11 patients have been treated under this program.

Of the cyclitis group 7/8 patients showed improvement, The anterior chamber activity quieted and cells in the vitreous decreased. Improvement of the retinopathy and visual acuity followed. Maintenance therapy at reduced dosages was continued in 4 patients with similar encouraging results. The longest patient follow-up is 20 months.

A striking improvement was observed in the treatment of a patient with sympathetic ophthalmia. This case was complicated by the presence of cataracts and glaucoma. Dramatic quiescence of his uveitis was noted after the initial injection of methotrexate. While on continued therapy cataract extractions were made possible and were successfully performed with almost normal restoration of vision. His glaucoma has since come under control without medication.

The results obtained in 2 cases of Bechet's were disappointing. Even when MTX was given in combination with high doses of steroids, relapses did occur.

Immunological Studies - The mononuclear phase of the inflammatory cycle in these treated patients was markedly suppressed. This occurred in the absence of leukopenia. The primary antibody response was likewise suppressed during therapy in all patients. Suppression of delayed-hypersensitivity and secondary antibody response could not be demonstrated.

It is concluded from these preliminary studies the anti-inflammatory effects of MTX were responsible for the observed improvement in these patients.

Significance of Program to Institute: The demonstration that an antimetabolite is effective in treatment of cases with uveitis is of practical importance. Information gained from such studies may also lead to a better understanding of the pathogenesis of uveitis.

Proposed Course of Project: To extend the present study to include other refractory forms of uveitis and to evaluate the feasibility of long-term treatment.

Part B Included: No

Serial No. NDB(I)-65 0/OC 1213(c)
1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Effect of Experimental Choroidal Ischemia on the Retina

Principal Investigator: Robert H. Collier, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 0.3
Professional: 0.3
Other: 0.0

Project Description:

Objectives: The purpose of this investigation is to determine the detrimental effects on the retina resulting from extensive choroidal ischemia without altering the retinal circulation or changing the normal chorio-retinal juxtaposition. It is the intent of this investigation to study both the acute and chronic effects on the retina by means of serial sections, ophthalmoscopy, and electroretinography.

Methods Employed:

(1) 20 cats have been utilized thus far. Of these animals, 8 were used to develop a satisfactory technique and 12 have been or are being studied as to the effect of the choroidal ischemia---a total of 40 eyes studied.

(2) The technique employed is an injection into a vortex vein of a thrombosing agent via a polyethylene canula.

Major Findings: The selective nature of this technique in producing choroidal ischemia without altering the retinal vasculature, in contrast to previous studies, permits one to investigate the role of the choroid in the nutrition of the retina and the functional inter-relation of the chorio-retinal circulation. The preliminary results indicate:

(1) With severe ischemia, the photoreceptors and their nuclei (the

layers of the retina external to the outer plexiform layer) appear to be completely dependent upon the choroid for their nutrition.

(2) With minimal ischemia, only the photoreceptors are affected with maintenance of the other elements of the retina.

(3) The pigment epithelium of the retina is affected in all of the eyes even with minimal ischemia.

Significance to Program of Institute: Several clinical disorders of the eye have been attributed to a change in the choroidal vasculature, but specific knowledge as to the function of the ocular choroid is lacking. As part of the institute's investigation of vascular disorders of the eye, this study is a beginning in the understanding of the role that the choroidal circulation plays in the nutrition of the eye.

Proposed Course of Project: Currently the acute stages of choroidal ischemia are being studied, but animals have been operated upon and are being maintained in order to study the long term effects of choroidal on the retina.

Part B included: No

Serial No. NDB(I)-58 O/OC 500(c)
1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Investigation of the Effects of Topically Administered Steroids on the Intraocular Pressure. Examination of Aqueous Humor Dynamics

Principal Investigator: George L. Spaeth, M.D.

Other Investigator: Ludwig von Sallmann, M.D.

Cooperating Units: National Institute of Mental Health
National Cancer Institute

Man Years (computed for the 12 month period)

Total:	2.4
Professional:	0.4
Other:	2.0

Project Description:

Objectives: (1) To investigate effects of topically administered corticosteroids on aqueous humor dynamics as measured by the intraocular pressure (IOP), on the response of the eye to a water load and on the coefficient of aqueous outflow, (2) to investigate the response of the normal and the glaucomatous patient to an oral water load, (3) to investigate the changes in intraocular pressure produced by rapid changes in systemic arterial and venous (induced by the Valsalva maneuver or similar procedure) and determine the possibility of such a procedure as a diagnostic test for glaucoma, (4) to investigate the interrelationships between intraocular pressure and autonomic nervous system functions.

Methods Employed: Dexamethasone 0.1% is instilled in the eyes (one eye at a time) of normal volunteers and patients. Data on the following functions are accumulated before, during and after periodic administration of the dexamethasone: Intraocular pressure, response of the eye to a water load (oral ingestion of 14 cc./kg. tap water - IOP before and every ten minutes for seven to ten determinations as measured with an applanation tonometer), the visual field, the size and responsiveness of the pupil. During most of the "water-drinking tests" blood is drawn at ten minute intervals for determination of the serum electrolytes and the total osmotic pressure; urine volume and specific gravity are also determined at the same time intervals. Variations in temperature, amount, concentration and route of administration are correlated with degree of effect on IOP.

The intraocular pressure was simultaneously recorded with skin resistance, heart beat (electrocardiograph), respirations (manometric), venous pressure (transducer in connection with a large bore indwelling needle), intrathoracic pressure (mouthpiece in connection with air pressure guage), and systemic blood pressure (Colson Blood) Pressure Recorder-utilizing sphygomometry). Intraocular pressure was almost invariably measured with a Muller electronic tonometer. A Gilson 8-channel polygraph was modified so as to accept the various inputs. Measurements of these variables were determined under conditions near to basal and then under several testing situations: (1) "cold pressor test," (2) forced expiration varying from 15 to 40 mm. Hg. pressure, (3) Valsalva Maneuver, (4) mental or emotional stress evoked by verbal questioning, (5) administration of topical medications in the eye - autonomic nervous system drugs and/or corticosteroids, (6) administration of systemic autonomic nervous system drugs (slow intravenous drip), (7) change of position by tilting by means of a standard tilt-table.

Laboratory determinations of serum electrolytes are made on all blood specimens drawn during water drinking tests. The total osmolality is determined by freezing point depression. The pH and PCO_2 are determined by routine techniques.

Visual fields are measured by Goldmann perimetry; tangent screen examination is also done where defects are primarily central in location.

Pupil size is measured with the scale on the Goldmann perimeter. No pupillographic studies have been done. Responsiveness of the pupil to various autonomic nervous system drugs administered topically is determined by noting the change in the size of the pupil.

In order to investigate possible effects of systemic adrenocorticosteroids four groups of patients are being studied: 1) psychiatric patients have their intraocular pressure measured daily by Schiotz tonometry. Urinary adrenal corticosteroid metabolites are determined and the IOP correlated with rate of excretion; 2) patients with systemic lupus erythematosis have periodic determinations of their intraocular pressure during their hospitalization - an attempt is made to determine the IOP prior to starting systemic therapy with adrenocorticosteroids and at various points during the therapy; 3) patients with hypersecreting adrenal carcinomas have their IOP measured prior to and after removal of their carcinomas.

The value of topical administration of corticosteroids as an investigative procedure is well established, but the period of instillation inconveniently lengthy (4-12 weeks). An attempt is being made to evaluate the effects of acute loads of topical steroids. Decadron 0.1% is instilled in one eye every five minutes for twenty doses; following this a standrad water provocative test is performed. IOP is measured before instillation of the decadron, immediately afterwards, and during the water drinking test. Pupil size is measured at 1 hour intervals.

Those patients on the Surgical Branch of the National Cancer Institute who are treated with radical neck dissections, during which the jugular vein(s) is (are) ligated have their intraocular pressure and outflow determined prior to and following their surgery.

Patient Material: The studies were conducted on glaucoma patients and normal volunteers admitted to the nursing unit of the Ophthalmology Branch of the NIMH. Included in the examinations were also outpatients with glaucoma, and inpatients admitted to the NHI, NCI and the NIAMD.

Major Findings: (1) Intraocular pressure is raised by topically administered corticosteroids. There patient depending upon whether they have "hereditary" open angle glaucoma, secondary glaucoma or no glaucoma. The first of these groups shows a rapid rise in intraocular pressure (within one week in most instances), the second a slower rise, and the last group shows a rise only after a more extended period of time, and then this rise is of smaller degree. The water drinking test is definitely altered in those patients experiencing a large rise in intraocular pressure, but is probably not changed when the response to steroid provocation is slight.

In approximately 10% of the cases the control eye (the eye not receiving topical corticosteroids) gave a more positive test following topical administration of the corticosteroids to the other eye than prior to such treatment.

The coefficient of aqueous outflow tended to decrease in most of the glaucoma patients as the intraocular pressure rose. The same was not true of the normal volunteers, and may indicate a different mechanism of action in the two groups. Similarly, the coefficient of aqueous outflow is decreased most noticeably in those patients in this present series manifesting the largest change in intraocular pressure. Mecholyl 2.5%, topically, produces a rise in intraocular pressure, in most eyes receiving topical corticosteroids. Epinephrine 0.1% and 2.0% produce a similar rise. These results are contrary to expectation and suggest that the topical steroids have altered the responsiveness of the eye to the autonomic nervous system.

(2) The studies on the osmotic considerations of the water drinking test are fairly complete. Results of 234 water drinking tests were analyzed. In 20% of the cases a

rise in intraocular pressure was noted prior to a fall in serum osmolarity. Changes in serum sodium and chloride paralleled those of the osmolarity. Thus, it was conclusively demonstrated that osmotic considerations are NOT the sole factor responsible for the rise in intraocular pressure that follows ingestion of a large water load. No significant change in pH occurred. The exact mechanism is not understood.

(3) When intrathoracic pressure is elevated quickly (as in a Valsalva maneuver), the venous pressure slowly rises to a peak and stabilizes at its new level. The intraocular pressure rises simultaneously, though not in an exactly similar fashion; it rises more quickly, more precipitously and independently of venous pressure (as measured in the antecubital vein). This can be demonstrated by having the volunteer exhale forcibly against a known resistance in a cyclical manner; when this is done the venous pressure will rise steadily and continuously whereas the intraocular pressure exactly mimics the pattern of changes in the intrathoracic pressure. Furthermore, while the venous pressure remains elevated as long as forcible exhalation is continued, the intraocular pressure rapidly decreases from its initial peak and then continues to decrease at a slower rate until it has returned to "normal" following which it continues to decrease at the rate characteristic of normal "outflow" as measured tonographically. In the glaucomatous patient the response is qualitatively different; here the initial rapid decline following the rapid rise is not seen. Some glaucomatous patients do not demonstrate the rapid rise. Though about 100 of these tests have been performed no clear cut picture has yet evolved. However, as testing technique improves the "usual" response is found more routinely in the normals, and various types of "unusual" response in the glaucoma patients.

(4) No positive correlations were found between skin resistance, pulse rate, and intraocular pressure. When subjects were stressed intraocular pressure changed only in those patients showing changes in respiratory pattern. It is more reasonable to ascribe changes in IOP to the changes in intrathoracic pressure than to systemic

autonomic effects, though these ANS effects have by no means been ruled out.

(5) Large doses of systemic adrenal corticosteroids given chronically or acutely have not resulted in detectable changes in intraocular pressure in patients in our study. No correlations were found in the psychiatric patients.

(6) Only two patients have had bilateral radical neck procedures during the past year. Intraocular pressure was elevated in both immediately post-operatively and returned to normal by the time of discharge. This study has not been entirely satisfactory because of the technical problem of measuring accurately the IOP of a patient just having had major neck surgery, for the head cannot be properly positioned. Patients with unilateral jugular ligations show a slight rise in intraocular pressure. The amount is within range of the unsatisfactory measuring technique.

(7) The plan to utilize subjects in Dr. M. Baldwin's physiological monitoring project has not materialized. So many technical problems have been encountered by the physician in charge of the mechanics of the study that only four tonographies have been performed. Hopefully this situation will improve and the study be continued.

Significance to Program of Institute: The response of intraocular pressure to administration of topical steroids may lead to a better understanding of those factors regulating the flow of aqueous and IOP.

It is still not understood why an oral water load produces a rise in intraocular pressure. It has been assumed that this is a relatively simple osmotic phenomenon.

The demonstration that rise in intraocular pressure consequent to oral ingestion of water is not solely the result of osmotic changes indicates that other factors, still completely undetermined, must be discovered. Venous pressure rose in a significant percent of patients given water loads, and this venous pressure rise may play a role in the IOP changes.

The response of the eye to topically administered adrenal corticosteroids may give us a clue as to the mechanism of glaucoma. Though it has been reported (Becker) that decrease in outflow is the first change and the major cause of the rise in IOP in patients given topical corticosteroids our studies do not support this contention. Studies being performed simultaneously by Dr. Macri tend to confirm the results of our investigation, namely that rise in IOP and change in outflow are not causally related.

It may be that one of the most important outcomes of this tudy will be re-evaluation of tonography itself. In our hands this procedure is, at best, only moderately repeatable. More important, tonography in vivo may not really be a simple measure of outflow but rather a qualitative pattern of response involving vascular, nervous and perhaps hormonal as well as mechanical considerations.

The role of venous and arterial blood pressure in regulation of the intraocular pressure is not well understood. If it were determined that these factors were important, it would indicate that the action of certain drugs is probably more on the basis of their effect on blood vessels than on the ciliary body; it would also indicate new areas of research into determination of the factors regulating the IOP. The diagnosis of early glaucoma is difficult; a diagnostic test superior to those now present would be of considerable aid in the management of patients. If it were determined that the autonomic nervous system played an important role in the regulation of intraocular pressure, new physiologic and therapeutic conception could be developed and investigated.

Proposed Course of Project: Sufficient information has been accumulated in most areas of study, and during the next months attempts to get this into finished reports will be made.

Further investigation into the possible mechanism of the water drinking test should be made; subjects should be given various blocking agents (atropine, etc.) prior to the tests and results compared with those of standard tests, animal experimentation with labeled water should be done.

Investigation of a new synthetic adrenal corticosteroid is planned. This substance (HMS-Allergan) is alleged not to cause a rise in pressure and yet to have potent anti-inflammatory effect.

Part B included: No

Serial No. NDB(I)-63 O/OPH 1018(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: A Study on Enucleated, Arterially Perfused Human and Cat Eyes

Principal Investigator: Frank J. Macri, Ph.D.

Other Investigators: None

Cooperating Unit: None

Man Years (computed for the 12 month period)
Total: 0.7
Professional: 0.3
Other: 0.4

Project Description:

Objectives: To use the presently available human and cat eyes for physiologic and pharmacologic studies.

Methods Employed: The eyes are perfused through the posterior ciliary artery or through the ophthalmic artery. Perfusate rate of flow and eye pressure are continuously measured. All eyes were perfused under identical conditions so that flow rates and eye pressures, where obtained, were more closely related to the individual eye rather than to variations in technique.

Major Findings: Cat and human eyes when perfused through the ophthalmic or posterior ciliary arteries have been considered viable in that they would respond to various pharmacologic agents. The importance of this preparation for studying the physiology and pharmacology of the eye without the complicating influences of a variable blood supply and an unknown nervous control is apparent. The "normalcy" of the preparation was therefore further explored.

Electroretinograms were made in five enucleated arterially perfused cat eyes and compared to similar ERG's obtained with the same eye in vivo. In all cases an ERG was elicited in vitro, in one case the "b" wave was positive (as was obtained in in vivo eyes); in 2 cases a depressed "b"

wave was obtained, then after one half hour reversed polarity; in the remaining two cases an initial finding of reversed polarity was obtained. In addition the rate of aqueous humor turnover and anterior chamber volume was determined in 20 cat eyes. Aqueous humor turnover rate was 65% of *in vivo* values and A.C. volume was 85%.

Considerable difficulty was encountered this year in obtaining freshly enucleated human eyes for study.

Significance to Program of Institute: The ability to provoke an ERG in enucleated arterially perfused cat eyes is quite encouraging. It indicates that the most sensitive portion of the eye is functioning; however, because of the inverted "b" wave a certain embarrassement to the circulation is indicated. Further evidence of physiologic viability was demonstrated by the ability of the eye to form aqueous humor. The decreased rate of formation, however, may be due to loss of nervous control or altered hydrostatic pressure in the perfused blood vessels.

Proposed Course of Project: It is proposed to continue these studies and to determine the effects of sympathetic and parasympathetic nerve stimulation on the rate of aqueous humor secretion. This data will help to define the role of the CNS in the regulation of secretion. In addition experiments will be performed to determine the effects of altering perfusate pressure. These experiments should help to clarify what if any role ultrafiltration plays in the formation of aqueous humor.

Part B included No

Serial No. NDB(I)-56 O/OPH 301(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Study on the Pharmacodynamics of Various Agents Affecting the Intraocular Pressure

Principal Investigator: Frank J. Macri, Ph.D.

Other Investigators: Rudolf Kern, M.D.

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 0.85
Professional: 0.45
Other: 0.40

Project Description:

Objectives: To determine the pharmacodynamics of agents effective in altering the intraocular pressure with a view toward finding more effective compounds and to possibly further the understanding of mechanisms which maintain the intraocular pressure.

Methods Employed: All the various agents investigated were examined on the cat eye. The preparations used were: (1) The intact living eye. (2) The arterially perfused enucleated eye. (3) The arterially perfused, isolated iris-ciliary body and iris preparations. The various functions measured were the intraocular pressure, iris artery pressure, and perfusate flow rate.

Major Findings: Previous studies performed in this laboratory have indicated that acetazolamide, in low doses, decreases the eye pressure not through the generally accepted mode of a decreased secretory rate but rather by decreasing intraocular blood volume. In order to further test these conclusions, which were based on blood flow and blood pressure measurements, the effect of acetazolamide on aqueous humor formation was studied. C^{14} labeled inulin turnover rates in the anterior chamber were used as a measurement of the inflow rate. The experiments were performed on anesthetized cats. 25 mg/kg doses of acetazolamide which have been demonstrated to lower the intraocular pressure were without effect on

aqueous humor formation. Much higher doses of this agent, however, did inhibit the formation of aqueous. The degree of inhibition at this high dose level was related to the dose.

Ouabain has been reported by some to decrease intraocular pressure while others have been unable to reproduce this finding. The mechanism postulated for the hypotensive response was inhibition of aqueous humor inflow, a concept which was based on indirect evidence. This question was studied by the inulin-C^{14} turnover methods. Three dose levels of ouabain were used, each of which decreased the rate of aqueous humor formation. The degree of inhibition was related to the dose. The dose required to produce 50% inhibition of aqueous humor production was very toxic causing 5 deaths in the 16 experiments performed.

Chlorothiazide, a diuretic with very weak carbonic anhydrase inhibiting properties, was also tested for its effect on aqueous humor formation. This drug has been reported not to influence the IOP or the dynamics of aqueous humor production and therefore was selected to serve as a control. Doses of this agent up to 600 mg/kg had, indeed, no inhibitory effect on the production of eye fluid.

Betamethasone has been shown to cause an increase in eye pressure of humans and was studied here to determine whether it acted on the vasculature of the eye. Studies on isolated, arterially perfused irides demonstrated that betamethasone (0.1 to 1.0 mg/l) was a smooth muscle depressant, preventing the vasoconstriction induced by such agents as eserine, ouabain, arterenol and acetazolamide. Extrapolating this data to the human situation, one would predict that neurogenic or humoral tonus exerted on these arteries would be lessened or abolished by this agent. Enucleated intact eyes were also perfused with betamethasone and in each case the eye pressure rose to high levels. Acetazolamide and ouabain were ineffective in reducing the IOP.

Mechanism of Action of various iris artery vasoconstrictors. We have reported previously that sympathomimetic agents (epinephrine, norepinephrine) parasympathomimetic agents (eserine, pilocarpine, BC-48, D.F.P.), histamine, ouabain and acetazolamide were all effective in constricting the iris artery. The similarity of responses is difficult to understand in view of the known diverse pharmacologic actions of these agents. Attempts were therefore made to determine whether all of these agents acted by a single mechanism or whether they acted through mechanisms already well known for these drugs. The action of eserine, pilocarpine, BC-48, and D.F.P. were inhibited by atropine but not by hexamethonium or priscoline indicating that these agents constrict by stimulating cholinergic, postganglionic receptors. Epinephrine and norepinephrine were inhibited by priscoline but not by atropine or hexamethonium indicating that they stimulated postganglionic sympathetic receptors. Histamine effects were abolished by pyrilamine and diphenhydramine but not by priscoline, atropine or hexamethonium showing the specific histamine response. Ouabain and acetazolamide responses could not be ihibited by any of the above blocking agents thus demonstrating that the action of these two agents is probably directly on the smooth muscle fibers.

Significance to Program of Institute: The finding that doses of acetazolamide which are sufficient to lower the IOP has no effect on aqueous humor inflow is an important departure from current concepts on the mode of action of this agent. Previously published reports from this laboratory have ascribed the activity almost solely to a vascular mechanism. This is supported by the lack of involvement of the secretory mechanism which many investigators felt caused the eye pressure to decrease. Ouabain, in the studies reported here, decreased aqueous humor formation yet there is no unanimity of opinion that it decreases the IOP. These data therefore lead one to wonder about the importance of secretion in the role of maintaining eye pressure (see also report 86(c)) and stresses once again the importance of the vasculature in this regard.

The pharmacodynamics of the various iris artery constrictors is still puzzling. It may be possible that the prototypes of the various groups act on different segments of the artery, but this would be difficult to demonstrate, experimentally. The similarity of response with all of these agents is in line, however, with the common eye pressure lowering effects that these drugs have in humans.

The smooth muscle depressant effect of Betamethasone is of marked interest for it can explain (see also project of Kern and Macri) all of the abnormalities noted clinically with its topical administration. It is important also in that it affords an experimental model for testing the efficacy of drugs in controlling eye pressure and could possibly explain the etiology of certain types of clinical glaucoma.

Proposed Course of Project: All avenues currently accepted to alter IOP have been explored in regard to acetazolamide and no further studies with this agent is anticipated. No further work is projected to clarify the bizarre similarity of iris vasoconstriction with the different classes of antiglaucoma agents until methods become available to assure that such an attempt would be fruitful. Further work with Betamethasone and similarly acting steroids is planned. Emphasis will be placed on their negative myotropic effect and a study on their effect on aqueous humor secretion.

Part B included: Yes

PHS-NIH
Individual Project Report
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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Macri, F. J.: The action of angiotensin on intraocular pressure. Arch. Ophthal. 73: 528-539, April 1965.

Honors and Awards relating to this project: None

Serial No. NDB(I)-59 O/OPH 600(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Role of Vasculature in the Maintenance of Intraocular Pressure

Principal Investigator: Frank J. Macri, Ph.D.

Other Investigators: Alberto Politoff, M.D.

Cooperating Units: Laboratory of Chemical Pharmacology, NCI

Man Years (computed for the 12 month period)
Total: 1.4
Professional: 1.2
Other: 0.2

Project Description:

Objectives: To determine if and by what mechanisms the dynamics of the intraocular vasculature may affect the eye pressure.

(a) The choroid plexus was studied because of its similarity in secretory function to that of the ciliary body. Measurements of potential across the epithelial layers of both the choroid plexus and ciliary processes were made. The objectives were to determine if potential gradients existed in these arterially perfused preparations which could help to better define the dynamics of secretion as well as to study the effects of secretory inhibitors.

(b) To determine if the blood vessels of the choroid plexus responded in a manner similar to the ciliary body.

Methods Employed: Lateral choroid plexuses of the cat, dog and rabbit were used. They were carefully removed and immediately perfused through either the anterior or posterior choroidal artery. Drug effects were ascertained by changes in the perfusate flow rate. Perfused anterior, middle and posterior cerebral arteries were studied as well as the hind limb, kidney, spleen and stomach.

Results: As with the iris artery, acetazolamide and norepinephrine produced a vasoconstriction of the vessels of the choroid plexus. Neither of these agents was effective in constricting the cerebral arteries. Eserine, ouabain, histamine and serotonin constricted the blood vessels of both areas. Only acetazolamide and norepinephrine were tested in the perfused organs (hind limb etc.). Acetazolamine was ineffective in these preparations, however norepinephrine produced characteristic vasoconstriction.

The determination of membrane potentials of the choroid plexus and ciliary processes were attempted. Micropipettes were used with appropriate amplification and paper recording. Potentials were obtained which varied generally between 8-15 mv, however they occurred only as transients. With the technique used, steady potential gradients could not be measured. No serious attempts were made to determine drug effects on these transients since we are primarily interested in potential gradients which are stable and may represent the gradients involved in secretion.

Significance to Program of Institute: A second structure, the choroid plexus has been found whose blood vessels constrict with acetazolamide. Other organs studied were unaffected. This is a significant finding in that the vessels in the two organs which are reported to be primarily secretory in nature are constricted with acetazolamine and both of these agents are known to decrease the rate of secretion of these organs.

The effects of drugs on cerebral blood flow has in the past been determined in a gross manner. In this study individual vascular beds have been perfused and their pharmacology studied.

The activity of a number of agents has been determined on these preparations which may be of physiological and clinical significance. In addition it has been demonstrated that not all of the brain arteries are equally responsive. These data may help in the understanding of problems in which the vasculature of the brain is felt to be involved.

The determination of potential gradients across the choroid plexus and ciliary processes and the effect of secretory inhibitors on this gradient would be helpful in indicating the secretory function of these tissues.

Proposed Course of Project: Further attempts will be made to obtain steady state potentials on the choroid plexus and ciliary process.

Drugs, other than those reported here, will be tested for their effects on the brain arteries so that further comparisons can be made with the eye.

Part B included: yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Macri, F. J.: The intraocular and vascular pressure of the cat eye. Exp. Eye Res. 3: 123-156, Sept. 1964.

Honors and Awards relating to this project: None

Serial No. NDB(I)-65 O/OPH 1214(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The In Vitro Effect of Corticosteroids on the Intraocular Muscles of the Cat and Monkey

Principal Investigator: Rudolf Kern, M.D.

Other Investigator: Frank J. Macri, Ph.D.

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 0.85
Professional: 0.45
Other: 0.40

Project Description:

Objectives: To determine the effects of corticosteroids on the intraocular muscles of the cat and monkey.

Methods Employed: Sphincter, dilator or ciliary muscle strips are dissected from animal eyes according to the technique of van Alphen. Two autologous strips are suspended in a muscle bath in oxygenated Krebs-Ringer solution and the effect of pharmacological agents on the tension in the strip is simultaneously measured by a strain gauge under quasi-isometric conditions.

Major Findings: Betamethasone at a concentration of 10^{-5} g/ml bath solution depresses remarkably (>90%) the effect of test drugs, such as acetylcholine, l-epinephrine, l-norepinephrine or serotonin on the sphincter pupillae, dilator pupillae or ciliary muscle of the cat and monkey. The latent period of Betamethasone is much longer than that of blocking agents, such as atropine, dibenzyline, etc. Furthermore, the response to agents, which depolarize the cell membrane, such as 40 mM KCl in isotonic solution, is depressed similarly. After careful washing, the muscles recover almost completely (latent period 30 min. to 2 hours, depending on the time of exposure to Betamethasone).

There are indications that the α-receptors of the sphincter pupillae are less resistant to the depressant effect of decreased dosages of Betamethasone (10^{-6} mg/ml bath solution) than the α-receptors of the dilator pupillae. Stimulation of the β-receptors of the ciliary muscle, on the other hand, induced an increased relaxation after exposure to Betamethasone.

It has been shown that Betamethasone reversibly decreases the basal tone of the tested intraocular muscles.

Significance to Program of Institute: Ptosis, slight mydriasis, accommodation difficulties, decreased facility of outflow and glaucoma are mentioned among others as side-effects of corticosteroids on the human eye. The in vitro results (see also report Dr. Macri/Dr. Kern), obtained from isolated intraocular muscles of the monkey, may partially explain some of these side effects. The results, elicited from isolated intrinsic muscle strips of the cat, are almost identical with those of the monkey, indicating that the depressant - but reversible - effect of Betamethasone seems to be a non-receptor-specific, non-species-specific one.

Proposed Course of Project:

(1) To study the action of other corticosteroids especially of those reported to have no effects on intraocular pressure.

(2) To determine whether this negative myotropic effect is specific for the intraocular smooth muscles, or whether the effect is non-organ-specific.

(3) To study the action of Betamethasone on the sodium-potassium "pump" of the cell membrane.

Part B included No.

Serial No. NDB(I)-65 O/OPH 1215(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Studies on Extraocular Muscles. (a) A Comparative Pharmacologic - Histologic Study of the Extraocular Muscles of the Rabbit. (b) The In Vitro Effect of Sympathomimetic Agents on Extraocular Muscles of the Cat, Monkey and Rabbit.

Principal Investigator: Rudolf Kern, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 1.25
Professional: 0.65
Other: 0.60

Project Description:

Objectives: (a) To study the tonic function of the Felderstruktur or slow fibres and to determine quantitatively the distribution of slow and twitch fibres in the different extraocular muscles of the rabbit. (b) To determine the presence and the nature of adrenergic receptors in the extraocular muscles of the cat, monkey and rabbit.

Methods Employed: For in vitro pharmacological studies the superior rectus muscle was carefully prepared in situ under the dissecting microscope, to obtain muscle strips consisting exclusively of the Felderstruktur (or slow) and others predominantly composed of the Fibrillenstruktur (or twitch) type. The strips were suspended in baths containing oxygenated Krebs-Ringer solution. The effect of acetylcholine on the tension in the two types of strips was measured by a strain gauge under quasi-isometric conditions. In order to determine the quantitative distribution of slow and twitch fibres in the different extraocular muscles, all four recti and the two oblique muscles of the rabbit were dissected from anesthetized animals and suspended in a similar way. The drugs used for the dose-response-curves were acetylcholine, d-tubocurarine and atropine. The

methods employed for the determination of the effect of sympathomimetic agents (part b) remained unchanged, but the study was extended to the extraocular muscles of the cat and monkey. The drugs used in the pharmacological assay were acetylcholine, l-epinephrine, l-norepinephrine, isoproterenol, dibenzyline, priscoline, DCI, alderline, d-tubocurarine and atropine.

Muscle preparations to be used for histologic studies were fixed in situ. The material was embedded in paraffin and cross-sections were subjected to various staining procedures. Divided muscle strips were processed similarly to the intact preparations.

Major Findings: (a) In the superior rectus muscle of the rabbit a superficial thin layer of small muscle fibers of the Felderstruktur type is separated by a fine leaf of richly vascularized connective tissue from a deeper, much thicker layer of large fibers of the Fibrillenstruktur type. In some respects the Felderstruktur fibers morphologically appear to be intermediate between striated and smooth muscle. The Felderstruktur preparations responded to acetylcholine (.05 µg/ml) with a tonic contraction, whereas the Fibrillenstruktur fibres, which comprise approximately four fifths of the bulk of the muscle, were either refractory to this dose of acetylcholine or reacted transiently and slightly (explained histologically by the admixture of "slow" fibres in the Fibrillenstruktur muscle plate).

Acetylcholine dose-response-curves demonstrate significantly ($P < .001$), that acetylcholine is two to three times more potent on the oblique than on the recti muscles of the rabbit, a fact which can be explained histologically by a larger number of Felderstruktur fibers in the oblique muscles. Increasing concentrations of d-tubocurarine and atropine, added to the baths, indicate that there is no qualitative pharmacological difference between the Felderstruktur fibres of the oblique and recti muscles of the rabbit.

(b) The results of studies on the in vitro effect of sympathomimetic amines on the extraocular muscles of the cat, monkey and rabbit indicate that the recti and oblique muscles of the rabbit and cat carry both α- and β-receptors (the rabbit predominantly of the α-, the cat of the β-type) whereas the adrenergic receptors in the same muscles of the monkey are almost exclusively of the β-type. The observations on dissected superior recti muscles of the rabbit suggest that the Felderstruktur fibres carry the adrenergic receptors.

Significance to Program of Institute: It was suggested by several authors that the Felderstruktur fibres in the extraocular muscles maintain the muscle tone. The present study established this function of the Felderstruktur fibres in the rabbit's extraocular muscles. It is possible that the tonic function of the "slow" fibres in the rabbits extraocular muscles is not limited to this species but operates in the extraocular muscles of all mammals in a similar manner, since there are no physiological or histological differences between the two types of fibers between species.

Saccadic eye movements are unusual in rabbits, although the extrinsic eye muscles are well developed. For the very slow rotary movements the two oblique muscles may play a predominant role.

It is perhaps of interest, that the distribution of the adrenergic receptors in the extraocular muscles and in the ciliary muscle - muscles, which arise from the same mesodermal tissue - is very similar within the same species. This study might be a small contribution to the pharmacology and pathology of the extraocular muscles, thus explaining - partially - the mechanism of some early stages of exophthalmus or enophthalmus.

Proposed Course of Project: To carry out electrophysiologic studies on single Felder- and Fibrillenstruktur fibres.

To study the dose-response-curves and the morphology of the extraocular muscles of other species.

To study the action of adrenergic agents on the extraocular muscles of other species.

Part B included No

Serial No. NDB(I)-65 O/CH 1216(c)
1. Ophthalmology Branch
2. Section on Cytology and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Thioacetamide-Induced Cataract in Trout

Principal Investigator: Ludwig von Sallmann, M.D.

Other Investigators: Patricia Grimes, B.A.
Eleanor M. Collins

Cooperating Units: Western Fish Nutrition Lab., Bureau of Sport Fisheries and Wildlife, Fish and Wildlife Service, U.S. Department of Interior

Man Years: (computed for the 12 month period)
Total: 0.15
Professional: 0.15
Other: 0.0

Project Description:

Objectives: To study the pathogenesis of cataract induced in trout by prolonged feeding of a standardized diet containing the carcinogenic agent, thioacetamide.

Methods Employed: Young trout maintained at the Fish Bioassay Laboratory, Hagerman, Idaho were fed a complete test diet plus 30 mg thioacetamide per 100 g. of dry ingredients for a period of 11 months in an attempt to produce hepatomata, as it is possible to do in rats. Cataract was observed in many of the experimental animals. A group

of live trout were shipped to Bethesda for biomicroscopic and histologic examination. Because many of the treated fish died in transit, other groups were examined biomicroscopically in the laboratory in Hagerman. The eyes were prepared for histologic study and cytologic examination of flat mounts of the lens epithelium. The eyes of additional fish, reserved for histochemical work, were rapidly fixed in liquid nitrogen - isopentane. Normal trout of the same age were examined and served as controls.

Major Findings: The experimental group consisted of 106 fish. After 11 months on the thioacetamide diet 95 of these had lens opacities. The cataracts were bilateral in 87 of the 106 trout. Biomicroscopically, the milder changes involved the anterior lens cortex and may have originated from an anterior polar opacity. The advanced cataracts involved most of the cortical layers of the lens.

Histologic examination demonstrated in many of the lenses a circumscribed proliferation of the lens epithelium in the region of the anterior pole. These cells form a plug which invades the underlying, apparently normal, cortical fibers. At the deep borders of the plug the epithelial cells degenerate. Preliminary results suggest that the proliferation of the epithelium is the primary event and that the anterior cortical cataract results from disruption of the fibers by the proliferating epithelium.

Significance to the Program of the Institute: Induction of liver tumors by feeding thioacetamide containing diets to rats has been studied in several laboratories for the past eight years. Lens opacities have not been reported in this species. Cancer of the lens has never been observed *in vivo*, but I. Mann demonstrated the malignant transformation of mouse lens epithelium and the development of infiltrative tumors when injured lenses plus a carcinogenic agent were implanted subcutaneously in inbred mice. If the epithelial proliferation observed in the thioacetamide-fed trout is the primary event in the development of cataract, it is possible that this is the first observation of a lens tumor produced *in situ* by a carcinogenic agent.

Proposed Course of the Project: To extend the study to rats when the investigation of the fish material is completed and for this purpose to utilize the techniques established for the rat lens as an experimental system.

Part B included: No

Serial No. NDB(I)-61 O/CH 801(c)
1. Ophthalmology Branch
2. Section on Cytology and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Autoradiographic Studies on Cellular Proliferation in Ocular Tissues

Principal Investigators: Ludwig von Sallmann, M.D.
Patricia Grimes, B.A.

Other Investigators: None

Cooperating Units: None

Man Years: (computed for the 12 month period)

Total:	2.05
Professional:	1.35
Other:	0.70

Project Description:

Objectives: To study the action of various cytotoxic and cytostatic agents on the proliferation of cells in the lens epithelium, in an attempt to compare the mode of attack of such agents, and to determine the effect of specific types of epithelial damage on the formation of normal lens fibers.

Methods Employed: Studies of the effects of triethylenemelamine (TEM) on DNA synthesis, cell division, and cell death in the lens epithelium have been continued using previously described methods.

The effects of chronic administration of myleran on the cell population of the lens epithelium were re-evaluated on the basis of the results of previous studies of changes produced by a single injection of the drug. For this purpose a group of young male rats of the Osborne-Mendel strain (100 g.) were fed a diet containing myleran at a level known to be cataractogenic (15 mg/kg diet). Food consumption and body weights of the treated and control animals were recorded regularly and animals were killed at selected intervals after the start of the experiment. The number of mitoses and disintegrated nuclei were counted in flat mounts of the lens epithelium, and the extent and degree of drug-induced polyploidy was determined. DNA levels of individual Feulgen-stained nuclei were measured with an integrating microdensitometer.

A study of the effect of age upon the diurnal variations in mitosis and DNA synthesis in the epithelial population has been started. Six-week, three-month, six-month, and one-year old male Osborne-Mendel rats were given an injection of H^3-thymidine (1 uc/g. body weight) and were killed one hour after tracer injection. Administration of H^3-thymidine was scheduled so that animals from each age group were killed at 6 A.M., noon, 6 P.M. and midnight. Autoradiographs of the Feulgen-stained lens epithelium were prepared and diurnal changes in the mitotic and H^3-labeled cell counts were compared in the different age groups.

Major Findings:

TEM: An earlier report described the effects of TEM on mitotic activity in the lens epithelium. It had been observed that single injections of the drug at doses of 1 and 2 mg/kg produced an inhibition of mitosis which reached a minimum of 24% of control values at 12 hours after injection. Recovery occurred during the second day and was characterized by an overshoot in mitotic activity. Investigation of the effect of TEM on DNA synthesis demonstrated that in contrast to the decrease of mitotic activity the number of cell nuclei labeled with H^3-thymidine more than doubled during the first 24 hours following administration of the drug. In the 1 mg group the increase of the number of radioactive nuclei was first seen at 8 hours. The counts

climbed to a peak value of 220% above the control levels at the 16 hour interval. They gradually returned to the normal range within one week. In the 2 mg group the rise of the number of labeled cells was delayed until the 16 hour interval, but the counts at 24 hours were as high as those obtained with the 1 mg dose. During the period when the number of synthesizing cells was high there was marked reduction in the amount of label per cell. The average number of exposed silver grains per nucleus was lowered to about 50% of controls. The grain counts remained approximately at the same level from 4 to 24 hours regardless of the injected dose, and all zones of the epithelium were affected in a similar fashion. This observation pointed to a reduced rate of precursor uptake and therefore to a reduced rate of DNA synthesis. The rise in the number of radioactive nuclei may be explained by a prolongation of the S period due to a slowdown in synthesis. No long-lasting effects of TEM at these dose levels were observed. Following the first week after injection and up to eight weeks the mitotic activity and the number of H^3-labeled cells was only slightly lower than in control animals and signs of nuclear disintegration were minimal.

Myleran: An earlier report from this laboratory described the effect of a single injection of Myleran (12.5 mg/kg) on cell proliferation in the rat lens epithelium. It was concluded that the drug acts in the relatively long G_1 period of the cell cycle in a manner which permits the cells to initiate and complete DNA synthesis normally but prevents their subsequent mitotic division. A portion of the arrested cells undergo nuclear fragmentation and death and the remainder return to interphase with a tetraploid level of DNA. The injury induced by a single injection of myleran seems to affect only one cell cycle, and the integrity of the population is eventually restored.

As expected, continuous administration of the drug in the diet led to a more extensive damage of the lens epithelium. The disruption of the cell cycle, however, was similar to that produced by a single injection. Mitotic activity fell gradually during the first week on the drug diet. Simultaneously, nuclei with aggregated chromatin appeared and increased to great numbers indicating an arrest in the

normal progression of cells to mitosis. Nuclear disintegration was marked by the end of the first week. After two weeks of myleran feeding, mitotic activity remained depressed, there were great numbers of "pre-prophase" nuclei, and, in addition, large tetraploid nuclei were seen. This pattern of events corresponded closely to those observed following a single dose of the drug. Subsequently, failure of cell division and cell death led to almost total disappearance of the equatorial and pre-equatorial zones. Mitotic activity remained markedly depressed up to eight weeks, the last interval studied. By four weeks a number of octaploid nuclei appeared among the remaining cells of the germinative zone, and at six and eight weeks cells of even higher ploidy were seen. It is apparent then that in the continuous presence of myleran a single cell may pass through several DNA duplication cycles without mitotic division thereby accumulating higher and higher levels of DNA.

Diurnal Variations in DNA Synthesis and Mitosis: Certain changes in the ratio of H^3-labeled cells to mitoses have been noted in the normal rat lens epithelium with increasing age of the animals. This observation suggested that diurnal fluctuations in proliferative activity may be intensified as the organism ages. Preliminary results of a study of diurnal variations of mitosis and DNA synthesis in animals varying in age from 6 weeks to 1 year indicate that the extent of the fluctuation of mitotic activity does not change. Although the number of mitoses per preparation decreases markedly with age, the number of mitoses during the period of highest activity was 1.8 times greater than during the period of lowest activity in 1 year old rats as well as in 6 week old rats. However, there appears to be a time shift of the periods of maximum and minimum activity. Six week old rats demonstrate greater mitotic activity at midnight and 6 A.M. and significantly less at noon and 6 P.M. Rats 3 months of age or older show a 6 hour shift of peak activity: the highest number of mitoses are seen at 6 A.M. and noon and the lowest at 6 P.M. and midnight. The corresponding counts of the number of H^3-labeled cells have been completed only in the 1 year old group of animals. At this age there is a marked fluctuation of DNA synthesizing cells of nearly the same magnitude as that of mitosis but approximately 12 hours out of phase. The periods of maximum mitotic activity correspond to periods

in which the number of DNA synthesizing cells is at a minimum. This phase difference may be explained on the basis of the timing of events in the cell cycle by which the initiation of DNA synthesis precedes mitosis by approximately 12 hours.

Significance to Program of Institute: Anti-neoplastic agents are used in ophthalmology for treatment of eye tumors and, recently, for possible suppression of autoimmune responses. Information on the mechanism of action on a cellular level, therefore, is of great interest. The lens epithelium is an excellent cell system for the study of the effects of these and other agents on the different phases of the life cycle of the cell. Thorough knowledge of the population dynamics of the lens epithelium, including age dependent changes in diurnal variations of the renewal process is important to permit meaningful comparison of results from various laboratories and to promote a better understanding of cataractogenesis.

Proposed Course of the Project: The investigation will be extended to the study of cytostatic or cytotoxic effects of drugs other than those studied so far such as, cyclophosphamide and certain pyrimidine analogues.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Grimes, P., von Sallmann, L., and Frichette, A.: Influence of Myleran on cell proliferation in the lens epithelium. Invest. Ophthal. 3: 566-576, December, 1964.

von Sallmann, L.: Cytologic studies on lens epithelium. A comparison of effects of X-rays, Myleran, and TEM. Invest. Ophthal. (In press).

von Sallmann, L., and Grimes, P.: Some effects of anti-cancer drugs on cell proliferation in the eye. Proceedings of the 2nd European Congress of Ophthalmology, Vienna, 1964. (In press).

Serial No. NDB(1)-63 O/OCH 1017(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Physical Chemistry of Corneal Collagen

Principal Investigator: Marc S. Lewis, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 1.5
Professional: 0.7
Other: 0.8

Project Description:

Objectives: To determine the physical and chemical parameters related to the molecular structure of corneal collagen with the view of examining these for significance in the morphology of the cornea.

Methods Employed: Collagen has been extracted from the bovine cornea, purified, and fractionated on the basis of salt solubility. These preparations have been studied by amino acid analysis, analytical ultracentrifugation, viscometry, and optical rotation.

Major Findings: A collagen has been isolated which not only appears to be monomeric, but has minimal intramolecular cross linking. Calculated dimensions based on the molecular weight, sedimentation coefficient, and intrinsic viscosity support the values obtained previously with polymeric preparations. Optical rotatory studies of the helix to random coil transition occurring with thermal denaturation showed that this preparation had a transition zone of width characteristic of collagens from other sources and unlike the very narrow transition zone of the polymeric corneal collagen. This implies that the structural stability of the polymeric form is very probably attributable to both intramolecular links between the sub-unit chains and intermolecular cross-links between the monomeric collagen molecules forming the polymer.

Significance to Program of Institute: This project represents a continued study of the relationship of the molecular structure of certain

proteins of ophthalmic origin to the structure and function of the tissues of their origin. It provides a base for comparative studies of different collagens in the eye, and for studies of the role of collagen in corneal development and in corneal pathologies.

Proposed Course of Project: Studies on the subunits of corneal collagen are planned, with particular emphasis on attempting to study the nature of the cross-link. Studies are also planned on scleral and vitreous body collagen. Studies on the development of collagen in the cornea of the chick embryo and on the collagen of the normal and pathological human cornea are now in progress.

Part B included: No

Serial No. NDB(I)-65 O/OCH 1217(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Physical Biochemistry of Model Gel Systems

Principal Investigator: Marc S. Lewis, Ph.D.

Other Investigators: Jules A. Gladner, Ph.D.
Albert J. Osbahr, Ph.D.

Cooperating Units: Laboratory of Biophysical Chemistry, NIAMD

Man Years (computed for the 12 month period)
Total: 0.4
Professional: 0.2
Other: 0.2

Project Description:

Objectives: To study the physical and chemical parameters related to the transparency and opacity of model gel systems. In this case fibrinogen was chosen as a model system because it will form either a transparent or an opaque gel when treated with thrombin depending upon the pH and ionic strength of the solution.

Methods Employed: Bovine fibrinogen and fibrin were modified by succinylation of the ε-amino groups of the lysine residues. The modified proteins were studied by analytical ultracentrifugation, optical rotation, and chromatography.

Major Findings: Succinylation increased the molecular weight of fibrinogen and fibrin to the extent of the succinyl residues added. When normal fibrin clots were dissolved in 5 M urea and succinylated, the material would no longer clot when the urea was dialyzed away. When succinyl fibrinogen was treated with thrombin it did not clot, the A peptide was released, the B peptide was not released, but a much larger fragment of the molecule (10,000 to 20,000 MW) was released. Optical rotatory dispersion data indicated very little loss in helical structure upon succinylation. From this data it was concluded that while marked changes in net charge and charge density had a limited effect on the susceptibility of specific bonds in fibrinogen to the action of thrombin, these changes apparently completely abolished the ability of thrombin to aggregate.

Significance to Program of Institute: This project represents one approach to the problem of understanding the physical and chemical aspects of gel formation which are pertinent to tissue transparency. At the same time, it represents an extension of information pertinent to the mechanisms of blood clotting.

Proposed Course of Project: Studies on the new subunit are being carried out by Doctors Gladner and Osbahr. Other studies are contemplated pertinent to the conditions which cause fibrinogen to form a transparent gel.

Part B included: No

Serial No. NDB(I)-65 O/OC 1218(c)
1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Ocular Manifestations in Carcinoid Syndrome

Principal Investigator: Vernon G. Wong, M.D.

Other Investigator: Kenneth Melmon, M.D.

Cooperating Units: Experimental Therapeutics Branch, NHI

Man Years (computed for the 12 month period)
Total: 0.2
Professional: 0.2
Other: 0.0

Project Description:

Objectives: To elucidate and correlate the ocular manifestations in patients with carcinoid.

Methods Employed: All patients were seen in consultations. Patients with interesting retinal findings were photographed or were otherwise drawn by medical illustration.

Major Findings: Ophthalmologically the carcinoid syndrome is manifested by periodic episodes of severe lacrimation and amaurosis fugax, conjunctival and periorbital edema and retinopathy.

The occurrence of lacrimation, amaurosis fugax and syncope is associated with the flush phenomenon which is strikingly characteristic of this disorder. The flush areas are generally confined to the upper half of the body where the skin areas assume a cyanotic hue at the height of an attack.

It was found that the ophthalmic artery pressures (as measured by the ophthalmo dynamometer) fell steeply whenever a flush attack occurred. In some patients this precipitous drop in pressure may have accounted for the temporary amaurosis and syncope. In most cases, the lowering of the intravascular pressure in the eye was associated with a generalized fall in systemic blood pressure.

The prodromal signs of the onset of a flush were severe lacrimation and marked injection of conjunctival vessels. This was seen in every

case examined and, therefore, could be used as a reliable index for the documentation of true flushes.

The retinopathy observed in these patients is of two types. The posteriorly situated lesions about the macula contain multiple retinitic spots and colloid bodies resembling drusen. In spite of these changes the patients had normal vision.

In the peripheral fundus a few patients showed discrete punched-out lesions with an incomplete rim of pigmentation around their borders. Adjacent venules to these lesions showed partial occlusion with pigment proliferation over their surface. The arterioles underwent marked attenuation with subsequent venous engorgement and blood sludging, during attacks of flushing. A case with prolonged flushing over a 4-5 day period developed multiple exudative lesions in the mid-peripheral fundus with occlusion of a branch vein. The resolution of the exudates resulted in punched-out lesions indistinguishable from those described previously. Proliferation of pigment over the occluded vessel eventually took place and subsequent IV fluorescein injection confirmed the absence of patency in the vessel.

The demonstration pf hyperserotonimenia is essential for the diagnosis of carcinoid. Recent isolation of a vasoactive peptide from several flushing carcinoid patients discounted the role of serotonin participating in such attacks.

It is felt, therefore, that the ocular manifestation of the carcinoid syndrome is flush induced. The extraocular changes of edema, injection, and excessive tear formation are probably related to the presence of bradykinin-like substances. And the production of the fundus pathology maybe the consequence of increased vascular permeability due to the effect of the peptide and repeated episodes of hypoxia induced by hypotension.

Significance to Program of Institute: Observations in these patients lend further support that changes in hemodynamics of the cardiovascular system and the presence of vasoactive substances can induce deleterious effects to the eye.

Proposed Course of Project: Plans to evaluate other carcinoid patients are continued. In the laboratory, studies of intraocular pressure in relation to flow changes induced by Bradykinin is being pursued.

Part B Included: No

Serial No. NDB(I)-65 O/OC 1219(c)
1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Ocular Complications of Chloramphenicol Therapy

Principal Investigator: Vernon G. Wong, M.D.

Other Investigators: Paul Lietman, M.D.

Cooperating Units: Pediatric Metabolism Branch, NIAMD

Man Years: (computed for the 12 month period)
Total: 0.2
Professional: 0.2
Other: 0.0

Project Description:

Objectives: Patients with fibrocystic disease of the pancreas and retrobulbar neuritis were studied clinically. Material from two of these cases were made available for histopathologic examination.

Methods Employed: Patients were seen through routine consultations with the Ophthalmology Branch at the Clinical Center. Also, a review was made of all records of patients seen at NIH with this disease who were not available for this study.

Major Findings: Six cases (all children under 7 yrs. of age) with retrobulbar neuritis were found in over 200 cases of cystic fibrosis of the pancreas. Five were males. In every instance the onset of visual impairment was sudden and bilateral central scotomas were found on examination. All these patients were on chloramphenicol therapy at the onset of their retrobulbar neuritis. Vision improved to normal in 4/5 patients when the antibiotic was discontinued. The 6th patient did not discontinue her medication because of her medical condition and no visual improvement was noted up to the time of her death. Optic disc pallor was observed on ante mortem examination of this patient.

The total dosage and duration of chloramphenicol therapy were found to be significantly greater in this group compared to other fibrocytics without optic neuritis. The total dose ranged from 87-274 gms. and the duration of therapy ranged from 3-1/2 mos. to 2 years.

Optic atrophy was diagnosed in 2 cases that came to autopsy. Marked

degeneration of the retinal nerve fiber layer, and demyelination and loss of axis cylinders of the optic nerve were observed in all cases. The lesions observed do not appear to be specific. The eyes of the brother of one of these patients were found to be normal histopathologically. He too died with cystic fibrosis.

Significance to Program of Institute: To make aware of occasional toxicity of prolonged chloramphenicol administration. The studied cases represent the largest number of patients with ocular complication of chloramphenicol reported to date with corroborated histopathologic findings in 2 patients.

Proposed Course of Project: No extension of the project is expected. It is anticipated that the above information will be helpful in the future management of patients receiving chloramphenicol therapy.

Part B Included: No

Serial No. NDB(I)-65 O/OC 1220(c)
1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Ocular Manifestation of Ehlers-Danlos Syndrome

Principal Investigator: William R. Green, M.D.

Other Investigators: Alvin Friedman-Kein, M.D.
William Banfield, M.D.

Cooperating Units: Dermatology Branch, NCI
Laboratory of Pathology, NCI

Man Years: (computed for the 12 month period)

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objective: To determine the ocular manifestations in the Ehlers-Danlos Syndrome.

Methods Employed: As many members of each family were examined when possible. In those members that were not examined, a history of signs and symptoms of the disease was noted. In most instances those patients that were studied were given complete general physical and ocular examinations. Other studies in these patients included the following: complete blood count, total platlet count, V.D.R.L., blood urea nitrogen, fasting blood glucose, cholesterol, total protein, albumin-globulin ratio, prothrombin time, coagulation time, Rumpel-Leed

test, and partial thromboplastin regeneration time. When permitted a skin biopsy was performed. Some of the skin specimens were studied by electron microscopy. Other studies on some of the patients included electrocardiogram, x-rays and chromosomal aberration studies.

Patient Material: The propositus in this study was first referred to the eye clinic with the diagnosis of central macular chorioretinitis. She was discovered to have the Ehlers-Danlos Syndrome and was studied as an inpatient. In a study of the family tree of this patient it was disclosed that there were 66 members of whom 15 were examined and found to have Ehlers-Danlos Syndrome.

A second family with the syndrome was on record in the medical records department. One member of this family had been studied previously at the Clinical Center. There were 52 members in this family of whom 18 were found to have Ehlers-Danlos Syndrome by history or physical examination.

Major Findings: The major finding in this study was the presence of angioid streaks in the propositus and the daughter of the propositus. In a review of the literature angioid streaks have not been reported in any patient with Ehlers-Danlos Syndrome alone. Other ocular findings were: blepharachalasis in two patients, poliosis in one patient, epicanthal folds in two patients and heterotropia in one patient. Chromosome aberration studies were negative. Routine and electron microscopic studies disclosed no change in the collagen or elastin that could be related to the Ehlers-Danlos Syndrome. Blood coagulation studies were normal in all patients tested. The Rumpel-Leed bleeding time was obtained in 10 patients with the Syndrome. Five patients had a positive test and all five were adults. The children with the Syndrome had negative Rumpel-Leed test.

Significance to Program of Institute: To our knowledge these are the first cases of Ehlers-Danlos Syndrome in which angioid streaks were associated without the presence of Pseudoxanthoma Elasticum. Although many first rate text books in ophthalmology have stated that

angioid streaks are associated with the Ehlers-Danlos Syndrome, the references in each case have been to three older articles in which angioid streaks and Ehlers-Danlos Syndrome were associated with Pseudoxanthoma Elasticum.

Proposed Course of Project: The study is to be written up including a review of the ocular manifestations of the Ehlers-Danlos Syndrome.

Part B included No

Serial No. NDB(I)-65 O/OC 1221(c)
1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Clinical Aspects of Fabry's Disease (Glycolipid Lipidosis)

Principal Investigator: George Link Spaeth, M.D.

Other Investigator: Phillip Frost, M.D.

Cooperating Units: Dermatology Branch, NCI

Man Years (computed for the 12 month period)

Total:	0.4
Professional:	0.4
Other:	0.0

Project Description:

Objectives: To delineate the clinical features of Fabry's disease.

Methods Employed: Standard clinical examinations and history taking were employed. In addition, histological, biochemical (thin-layer chromatography) and genetic aspects were studied. Complete blood grouping is being performed. Autopsy material from one patient has been obtained and studied.

Patient Material: The studies were conducted on 39 members of two families. One patient was admitted to the National Cancer Institute for in-patient studies.

Major Findings: A characteristic cataract, not previously described was noted. A peculiar distribution of skin telangiectasia was also a new finding. One patient was found to have histological evidence of Fabry's disease in the absence of skin or ocular changes; this has not been previously reported. A characteristic radiographic defect in the temporomandibular joint, hitherto undescribed, was documented. Electron microscopic examinations showed the presence of granules with a periodicity of 50-200 A, not well demonstrated before. Blood grouping studies are not yet complete.

Significance to Program of Institute: Fabry's disease is a member of the Glycolipid storage diseases (Tay-Sachs', Gaucher's). Specific localization of the enzyme defect is dependent upon supply of the specific metabolite involved. Such material should now be available. Clinical characterization of the disease has not been complete in the past.

Proposed Course of Project: Biochemical identification of material from the autopsy specimens.

Part B included: No

Serial No. NDB(I)-65 O/OC 1222(c)
1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Ocular Manifestations of Chlorpromazine and Other Phenothiazines

Principal Investigator: J. Brooks Crawford, M.D.

Other Investigator: None

Cooperating Units: Selected Mental Hospitals

Man Years (computed for the 12 month period)
Total: 0.2
Professional: 0.2
Other: 0.0

Project Description:

Objectives: Because a unique pigmentation of the lens, cornea and exposed skin of individuals receiving high and prolonged doses of chlorpromazine has been reported it seems advisable to:

(1) Attempt to reproduce this pathology experimentally in animals, thereby permitting further evaluation of the pathology and mechanisms involved.

(2) Determine the incidence of this pathology in patients receiving chlorpromazine.

Methods Employed:

(1) 18 pigmented rats (9 male and 9 female) have been receiving large doses of chlorpromazine orally mixed with their normal diet for 5 months. 6 animals (3 male, 3 female) are used as controls. All the animals are being examined periodically with the biomicroscope for evidence of corneal or lens changes.

(2) A group of patients at various mental institutions have been selected by their attending physicians for treatment with high doses of chlorpromazine in an attempt to control their psychiatric symptoms.

A protocol has been established for the ophthalmologic examinations prior to, during, and after therapy.

Major Findings:

(1) After 5 months of continuous high doses of daily oral chlorpromazine, none of the rats have developed corneal or lens changes.

(2) The project associated with selected patients at various mental institutions is just commencing and no reports have yet been obtained.

Significance to Program of Institute: It is important to determine if chlorpromazine causes eye pathology, the extent and nature of such pathology and the mechanisms involved.

Proposed Course of Project: If the rats which are receiving chlorpromazine do not develop corneal or lens changes, this part of the project will be terminated.

The results from the ocular examinations of mental patients receiving high doses of chlorpromazine will be correlated and evaluated.

Part B included No

Serial No. NDB(I)-65 O/CH 1223(c)
1. Ophthalmology Branch
2. Section on Cytology and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Studies on the Enhancement of Contrast in Isolated Particles and Thin Sections

Principal Investigator: Bolivar J. Lloyd, Jr.

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

Objectives: To investigate the effects of the vertical evaporation of varying small amounts of chromium on both isolated virus particles and thin sections in the electron microscope. To compare the enhancement of contrasts due to vertical shadow with conventional methods of positive and negative staining.

Methods Employed: An RCA electron microscope model EMU3F is used in these studies. A standard vacuum evaporator is used to vertically evaporate small amounts of chromium onto specimens at a distance of 18 centimeters. Specimens prepared by air drying isolated virus particles and thin sections of various tissues which have been prepared by the routine methods for electron microscopy using buffered osmium tetroxide as a fixative followed by dehydration in alcohol and embedding in Epon 812. Sections are cut with an LKB Ultratome and transferred to collodian filmed grids covered with a fine carbon layer.

Major Findings:

(1) Isolated virus particles: Differences in contrast of fine structure both on the upper surface and at the edges. The effects are similar to those of rotary shadowing. Tobacco mosaic virus which had

been isolated and allowed to air dry on filmed grids show these diferences in contrast in the electron microscope.

(2) Thin sections: Using vertical shadow, fine structure of 30-40A° is seen more plainly in the electron microscope. In the cornea of the eye of the grasshopper, sections which have been shadowed vertically showed fine structure of this order of magnitude better than the same material untreated. In lipid deposits which were found in the conjunctiva of tissues taken from a case of Fabry's disease, myelin figures of the order of 40A° are seen and the contrast is apparently enhanced by vertical shadowing.

When compared to conventional staining methods, vertical shadow is sometimes preferable, although each method has its own merits and conditions; where one is more productive than another must be determined.

Significance to Program of Institute: Using this method of contrast enhancement on ophthalmic tissues might yield some details of fine structure that would not be evident by any other method.

Proposed Course of Project: Further observations using various conditions will be continued with comparison to standard procedures and the study shall be prepared for publication.

Part B included: No

Serial No. NDB(I)-56 O/OPS 300(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

rt A

Project Title: Design and Construction of Ophthalmic Instruments; Research in Psychophysical Methods of Evaluating Vision

Principal Investigator: Ralph D. Gunkel, O.D.

Other Investigators: Peter Gouras, M.D.
Ronald Carr, M.D.

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 1.0
Professional: 1.0
Other: 0.0

Project Description:

Objectives: To make improvements or modifications of instruments used in clinical and experimental ophthalmological work. Specifically, to design and/or construct such devices as are suggested or required by current projects and to follow through in their use.

Methods Employed: In connection with the electroretinographic, d.c. potential and single unit measurements of Dr. Gouras, a great many ideas have had to be developed:

A new type microelectrode holder was designed and constructed of methyl methacrylate and later in nylon.

The photic stimulator of Eberhard Dodt was transferred to an adjustable table to permit greater flexibility in its use.

The old Bausch & Lomb fundus camera was modified to use monochromatic light from the photic stimulator for observation, stimulation, and photography of the retinae of monkeys while a microelectrode is inserted through an incision in the anterior sclera. The heavy camera was mounted on a swinging base so it can be easily removed from the working area around the monkey's head and returned rapidly to the same position.

Further modifications were made in the contact lenses to permit passage of the microelectrodes and to help minimize eye movements.

Several changes were made in the micromanipulator and a target projection device was constructed for stimulating specific retinal areas.

A Polaroid camera adapter was designed and constructed for photographing single or multiple electroretinograms as they appear on a special oscilloscope.

A suitable red-free filter combination was determined for improving the constrast and resolution of certain retinal details with black and white Polaroid film -- which can be developed in ten seconds.

Many tests of retinal sensitivity (profiles) were made, particularly in patients receiving chloroquine therapy. After extensive comparison of static perimetry with retinal profiles, it became apparent that the latter was the method of choice for early detection of damage to the macula or perimacular retina from chloroquine toxicity. This matter is covered in greater detail in the report of Dr. Ronald Carr.

The plotting of dark adaptation curves and retinal profiles continues as a routine diagnostic procedure in certain types of cases. In addition to this, an intensive series of tests was done in connection with the acanthocytosis study, also with Dr. Ronald Carr.

Significance to Program of Institute: Modifications in the electrophysiological apparatus have aided in the rapid progress of research in this field.

Retinal profile studies have contributed substantially to the chloroquine project, as have the dark adaptation measurements to the acanthocytosis study.

Some of the clinical associates have been motivated by the quick color Polaroid to take more serial photographs and in some cases to make informative collages of large retinal areas.

Commercial manufacturers have seen fit to make this particular Polaroid fundus camera adapter available to the profession.

The discovery of suitable filters for red-free retinal photography presents some interesting possibilities and may also be applied to the tracing of fluorescent materials in the ocular blood vessels.

Proposed Course of Project: It is proposed that this project be continued in its present flexible form.

Part B included No

Serial No. NDB(I)-63 O/OC 1019(c)
1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Effects of Topical Corticosteroids on Aqueous Humor Dynamics

Principal Investigators: John P. Nicholas, M.D.
Ludwig von Sallmann, M.D.

Other Investigator: Gerard van Alphen, M.D.

This project is terminated. Work has been incorporated with NDB(I)-58 O/OC 500 (c) - Investigation of the Effect of Topically Administered Steroids on the Intraocular Pressure. Examination of Aqueous Humor Dynamics (Spaeth and von Sallmann).

Serial No. NDB(I)-63 O/OC 1015 (c)
1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Intraocular Penetration of Amphotericin B

Principal Investigator: William R. Green, M.D.

Other Investigator: John E. Bennett, M.D.

This project is complete.

As a blood-brain barrier tracer 3 ml of 10% sodium fluorescein was injected prior to the application of the cold plate. The animals were sacrificed at varying time intervals and the brains were sectioned for gross observations and photography under the U. V. light.

Major Findings:

Elevation of the systolic blood pressure up to 200 mmHg resulted in a striking acceleration of the progression of the edema. Thus, the extent of the edematous area in cats with 200 B.P. sacrificed after 2 hours following cold lesion corresponded to that observed in animals under normal blood pressure, 10 hrs. after cold injury. Conversely, cats with systolic B. P. lowered down to 50 mmHg which were sacrificed 6 hrs. following the cold injury revealed a conspicuous inhibition in the spread of edema, the area outlined by the fluorescein tracer corresponding to that observed in normotensive cats 30 minutes after cold injury.

Proposed course of the project:

Further investigations concerning the effect of the systolic B.P. on the development of the brain edema will be concerned with the relationship between the duration of altered B.P. and the dynamics of edema. Particularly, an attempt will be made in assessment of the value of hypotension in controlling the course of edema, which might be of a considerable clinical significance.

Part B included No

Serial No. NDB(I)-63 O/OC 1013(c)
1. Ophthalmology Branch
2. Clinical Investigation
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Clinical Study on the Suppression of Pyrimethamine (Daraprim) Toxicity by Folinic Acid

Principal Investigator: Conrad L. Giles, M.D.

Other Investigator: None

This project is complete.

This is particularly true for the cerebello-cortical connection. Further, the long lasting facilitatory and inhibitory effects of the cerebellum upon single cell discharges from the ipsilateral and contralateral motor cortex further emphasize the importance of the cerebellum in motor function.

Proposed Course of Project: This is to be extended to the study of physiological connection of the cerebellum and various subcortical structures.

Part B included Yes

Serial No. NDB(I)-62 O/OPS 904(c)

1. Ophthalmology Branch
2. Section on Ophthalmology
   Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Analysis of Evoked Responses in the Human Visual System

Principal Investigators: Peter Gouras, M.D.
Ronald E. Carr, M.D.
Ralph D. Gunkel, O.D.

Other Investigators: None

This project has been terminated. The work is being continued and incorporated in projects NDB(I)-63 O/OC 1012(c) - Electrophysiological and Psychophysical Studies of Tapeto-Retinal Degeneration - Clinical and Experimental (Carr, Gouras, Gunkel, and Crawford) and NDB(I)-63 O/OPS 1016(c) - Electrophysiological Studies of Mammalian Retina (Gouras, Carr, and Gunkel).

Serial No. NDB(I)-56 O/CH 302(c)
1. Ophthalmology Branch
2. Section on Cytology and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Electron Microscopic Studies on Tissues of the Eye, Such as Epithelium, Fibers and Capsule of the Lens, and the Conjunctiva

Principal Investigator: Theodor Wanko, M.D.

Other Investigators: Ludwig von Sallmann, M.D.
Bolivar J. Lloyd, Jr., B.S.

This project has been terminated for the present time. Work will be started again when the new Head of the Section assumes his duties and the laboratory is reactivated.

of muscles, 3. those which discharge only with relaxation of certain groups of muscles, 4. those whose discharges are decreased with relaxation of certain groups of muscles and 5. those which are indifferent to contraction and relaxation of muscles. Furthermore, preliminary analysis with the computer indicates that there is a close cross co-relation in the activity of cells of the cortex and of the thalamus. Finally, bilateral representation of motor activity could be demonstrated by single cell discharges recorded from the cortex and thalamus.

Significance to Bio-Medical Research: This investigation may lead to a new field in which the integration function of the human brain at a cellular level could be studied in patients subjected to brain surgery.

Proposed Course of Project: It is proposed that this study be extended to the cerebellum which is known to be of great importance in motor function.

Part B included Yes

ANNUAL REPORT

July 1, 1964 through June 30, 1965

Branch of Electroencephalography and Clinical Neurophysiology, IR
National Institute of Neurological Diseases and Blindness

Cosimo Ajmone Marsan, M. D., Chief

Summary of Program Activity

1. Clinical-Diagnostic Service

This service represents a considerable portion of the overall activity of the Branch, over 30% of man years being devoted to it.

From March 31, 1964 to March 31, 1965 a total of 1,749 examinations have been carried out in the EEG Laboratory. These examinations were performed on both in- and out-patients (1,345 and 404, respectively), following referrals from the various Institutes in this order:

Institute	No.	%
NINDB	1025	58.5
NCI	216	12.3
NIMH	174	10.0
NIAID	139	8.0
NIAMD	107	6.1
NHI	88	5.1
Total	1749	100.0

The monthly average of EEG examinations (145) has slightly increased from that of the last report but remains fairly close to the monthly average for the last 6-7 years and to what appears to be a reasonable figure, considering the available facilities, the type of examination that is generally required and the needs of the various Institutes.

Most of these examinations are performed as a clinical service or as part of investigative projects in which this Branch (or Institute) has no direct interest; some of this activity, however, is also applied toward the fulfillment of projects in which this Branch cooperates and/or is primarily involved (see description of individual projects). This is true, for instance, in the case of the numerous studies (see Branch of Surgical Neurology) carried out in patients in whom electrodes have been chronically implanted for diagnostic-therapeutic purposes. In addition to these and to the routine EEG scalp examinations, records

have been obtained directly from the exposed cerebral cortex in the course of 27 surgical operations.

2. Research activity

Some of the projects already outlined in previous reports have been completed in the period covered by the present report. Among these the results of the microelectrode analysis of the nucleus gracilis of the cat were presented at the annual meeting of the American Electroencephalographic Society (Santa Fe, New Mexico, October 1964) and at that same meeting, this report was awarded the Hans Berger Prize as the best paper from a junior investigator. Among its findings are: a) the identification and distinction of pre- and post-synaptic elements in the nucleus; b) the relative specificity in the topographical (rostro-caudal) distribution of neurons responding to different sensory modalities; c) the relationship between extent of nuclear receptive fields and peripheral areas; d) the powerful influence (mostly "inhibitory") of the motor cortex upon the activity of over 50% of the elements of the nucleus and the suggestive topographical arrangement of these elements in relation to their different susceptibility to such corticofugal effects; e) the anatomo-functional distinction apparently existing between different portions of the nucleus.

The findings of another study related to project 1001(c) have also appeared recently in printed form. The project is related to one of the experimental forms of epilepsy, and deals with the effects (and their mechanism) of local cortical strychninization on various types of inhibitory post-synaptic potentials in neurons of the cat motor cortex. On the basis of suggestive results it has been possible to offer an alternative to the commonly accepted hypothesis. Thus strychnine, rather than interfering with the action of the inhibitory transmitter, could actually damage the neuronal membrane and, consequently, abolish the post-synaptic hyperpolarization or even convert it to a depolarizing potential.

A new project underway is the study of the electrographic correlates of the various phases of nocturnal sleep. This project involves the time-consuming procedure of collecting data during the entire night while selected patients, with a number of intracerebral electrodes, are in their natural sleep. It has long been recognized that the electrical activity of the brain goes through a series of different patterns which are apparently related to the different "depths" of sleep. More recently a sleep phase has been identified with well defined clinical and electrographic characteristics. This phase has been, and is currently the object of different forms of investigations in both animal and man. It is also a well known fact that various seizure disorders are variably influenced by the sleep-wakefulness cycle and that certain forms of epilepsy tend to be "activated" during sleep. The present patient material lends itself to the interesting study of this general problem of sleep in "normal" and epileptic

subjects. To date no definite results are available but the collection of data and their analysis continues and it should soon be possible to learn something about the role of various subcortical structures in, and their activity during, sleep as well as the possible mechanisms of nocturnal seizures.

Other projects have been started in the course of this fiscal year and deal with specific problems about the physical properties and the basic physiology of neurons. These projects are at different stages of investigation and none has been completed at the time of this report although the experimental phase of the work is over. Briefly, the main purpose is to obtain information about resistance, time constant and rectification properties of the membrane of neurons in the cat cerebral cortex. These data are studied in resting conditions and during synaptic impingement in the "normal" neurons as well as in neurons which have been affected by local strychninization. It is hoped that some of the data might help to understand the nature of the so-called spontaneous activity of the brain.

The analysis of the experimental data from these studies involves a long series of careful measurements and calculations. These are presently in course and the major findings which are mentioned in the project descriptions should be considered provisional.

A critical survey has been completed of the world literature in the field of epilepsy for 1964 (as well as the 1963 material which had not been included in the analogous study of the previous year). This review will form the chapter on "Epilepsy" for the yearly volume of "Progress in Neurology and Psychiatry". Over nine hundred papers dealing with various aspects and forms of seizure disorders, experimental convulsions, their experimental and clinical pharmacology, etc., have been surveyed. Besides the direct interest of both Branch and Institute in this field, this modest effort has required a relatively small investment in man-years and should prove of some practical usefulness in an era of over-publication which greatly hampers any individual attempt at bibliographic documentation. A theoretical-critical discussion has been published on the nature of the electrical activity of the brain. The various experimental data which might throw some light on the possible relationship between neuronal activity and "slow" waves of the EEG, have been analyzed and discussed.

3. Other activities, organizational aspects and miscellaneous information.

Training in clinical electroencephalography has been provided for two doctors. The type and extent of such training is felt to be adequate for a neurologist primarily interested in clinical work and simply wishing to broaden his practical knowledge in one related laboratory technique but it could hardly be considered sufficient for a person who plans to become a full-time, Board qualified electroencephalographer.

Some of the reasons which are more or less directly responsible for the limited usefulness and/or unsatisfactory results of this important part of this Branch activity have been outlined in the previous reports; but nothing could be done to modify the existing situation and, whatever the achievements, these are almost exclusively due to the efforts and efficiency of the technical staff and of the medical officer of the Branch.

Space remains a serious problem in the clinical Section of the Branch and a particularly acute one for the professional staff which has to work in rather unfavorable physical conditions. The same problem has also prevented the creation of a record library, an important facet of all EEG Laboratories. Due to lack of conveniently located storage space, records older than two years have to be mircofilmed and are subsequently destroyed. The microfilm has limited usefulness and a considerable amount of interesting material is no longer available.

The Branch Chief has continued his part-time activity as Chief Editor for American and the Far East of the International monthly Journal, "Electroencephalography and Clinical Neurophysiology". This challenging and interesting form of activity is considered rather rewarding and it is an activity which greatly contributes to the overall scientific standing of the Branch and, indirectly, of the Institute.

The Branch Chief is presently preparing the material for a number of Synposia and Discussions in which he has been officially invited to participate next September, on the occasion of the various International Congresses to be held in Vienna and Prague. Details of these presentations will be outlined in the next year's report.

The official positions of the Branch Chief include that of Delegate for the American EEG Society to the International Federation of Societies for Electroencephalography and Clinical Neurophysiology and that of Chairman of the Board of Qualification (in clinical electroencephalography) of the American EEG Society. He has recently been nominated to succeed Dr. M. A. B. Brazier as President of the International Federation of Societies for Electroencephalography and Clinical Neurophysiology but has refused this honor in view of the excessive time and activity this office would involve.

The Chief EEG Technician, Mrs. M. Berkeley, was chosen to be the second President of the recently formed American Society of Electroencephalograph Technicians, an honor she fully deserves.

4. Program considered for the near future.

The main investigative interests of the Branch will not shift significantly from those outlined in the present report.

Due to the particular organizational position of this Branch, projects of a primarily clinical nature have to depend almost entirely on the available patient material, or, in other words, they are determined by the current research interest of other Branches and Institutes. In this respect, a close and reciprocally beneficial cooperation is anticipated to continue with the Branch of Surgical Neurology of our Institute.

A certain amount of modernization of equipment is considered. This should require a slight transitory budget increase but also permit a broader or different technical approach to various problems related to the electrical activity of the brain in normal subjects and patients with CNS ailments. Epilepsy in its different aspects and manifestations, and especially its pathophysiology and diagnosis will continue as one of the main areas of investigation in both clinical and experimental fields. Neurophysiological research will probably remain centered around cortical phenomena and/or subcortical-cortical integration in mammals.

5. List of publications.

This list includes only papers related to projects described in previous reports but not yet published when the last report was prepared. It excludes abstracts, book reviews, papers in course of publication and those papers which are related to current projects. The latter, if available, are listed in part B of the respective project descriptions:

Pollen, D. A. Intracellular studies of cortical neurons during thalamic induced wave and spike. Electroenceph. clin. Neurophysiol. 17: 398-404, Oct. 1964.

Ajmone Marsan, C. Electrical activity of the brain: slow waves and neuronal activity. Israel J. Med. Sci. 1: 104-117, Jan. 1965.

Ajmone Marsan, C. and Abraham, K. Epilepsy, In E. A. Spiegel (ed.): Progress in Neurology and Psychiatry, New York, Grune & Stratton, 1964: pp 261-298.

Serial No: NDB(I)-61 EEG/OC 800(c)
1. Electroencephalography and Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Depth recording and stimulation in man.

Principal Investigators: C. Ajmone Marsan and J. Van Buren

Other Investigators: K. Abraham and C. L. Li

Cooperating Unit: Branch of Neurological Surgery, NDB

Man Years (NDB-EEG only)

Total:	1.2
Professional:	0.2
Other:	1.0

Project Description:

Objectives, Method, Significance, etc.: This project has two main purposes: a) to investigate the functional characteristics of various subcortical structures in man; b) to establish some diagnostic-localizing criteria of possible practical significance in the treatment of certain neurological disorders.

Method and patient material: The investigation is to be carried out on all those patients (from the Branch of Surgical Neurology) in which multiple-contact electrodes have been implanted and kept in place for a variable period of time prior to their therapeutic surgery. Patients are either epileptic or affected by involuntary movement disorders. Electrode implantation, number of leads and their localization within the brain is variable and depends on the individual clinical problems. The main portion of investigation as far as the EEG Branch is concerned, deals with the analysis of the numerous records which are obtained from each patient.

Major findings: Most of the accumulated records are still in the process of being analyzed. The data relating to seizure cases will form the object of a report which the principal investigator (C. Ajmone Marsan) has been invited to present at the Symposium on Stereoencephalotomy to be held in Vienna, Austria, September 1965.

Significance to the Program of the Institute: The project should help in throwing light on the type of electrical activity which characterizes different nuclear masses within the brain, and indirectly, on the reciprocal functional relationship as well as on their relationship with the cerebral cortex. The distribution of certain abnormal patterns, such as those occurring in seizure disorders should also help in determining the possibility of (while serving as a guide for) surgical therapy.

Proposed Course of Project: To complete the analysis of the data on epilepsy cases and to start that of data on records from patients affected by involuntary movements. The most immediate goal would be to assess the particular form of electrical activity which might characterize different subcortical structures.

Part B included: Yes ☐ No ☒

Serial No: NDB(I)-62 EEG/CN 901(c)
1. Electroencephalography
and Clin. Neurophysiology
2. Clinical Neurophysiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: N. gracilis of cat. Functional organization and corticofugal effects.

Principal Investigator: D. L. Winter

Other Investigators: None

Cooperating Unit: None

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives, Method, Significance, etc.: To study sensory representation of certain parts of the body surface at the level of the first relay nucleus of a major sensory system. This to be carried out at the unitary neuronal level within the nucleus. An additional object of investigation is the possible role of corticofugal influences upon the activity of the neural elements in the same nucleus.

Method: Acute experiments were carried out in lightly anesthetized cats using standard microelectrode technique to record the extracellular activity of single cells in the n. gracilis during various types and modalities of peripheral stimulation and also during electrical stimulation applied to the cerebral cortex.

Major Findings: Single units in n. gracilis of cat were activated by physiological and peripheral electrical stimulation and their activity was tested after a conditioning stimulation of the motor cortex with the following findings:

1. Presynaptic and postsynaptic units could be distinguished within the nucleus on the basis of a number of different criteria. About 40% of the total unit population was presynaptic.

2. The distribution of postsynaptic units responding to specific physiological stimuli was analyzed throughout the rostral-caudal plane of the nucleus. There were significantly fewer units responding to hair movement and significantly more units responding to joint movement in the rostral part of the nucleus as compared to the middle and caudal portions. Units responding to touch and deep stimulation were distributed evenly throughout the nucleus.

3. The sizes of the receptive fields, determined for presynaptic units responding to hair movement in hind limbs and trunk, were found to be related to the specific peripheral area and tended to increase in a distal-proximal direction.

4. Receptive field sizes of postsynaptic units responding to hair movement were determined. As in the case of presynaptic units, they were found to be related to that portion of the extremity that they represented. No significant differences were found when fields from any given peripheral area were compared by location of recording site within the nucleus.

5. The activity of 53% of all postsynaptic units tested was modified by rostral stimulation in one or more of the following ways: activation, facilitation, postspike depression, and inhibition.

6. Ninety-one per cent of the modified units were inhibited, regardless of previous activation by the conditioning stimulus. Forty-seven per cent of the modified units were initially activated. Only ½ these units showed postspike depression. Forty-five per cent of the modified units exhibited both excitatory and inhibitory effects.

7. The distribution of modified cells was not homogeneous throughout the nucleus. Seventy per cent of units in the rostral portion and 30-40% of units in the middle and caudal portion of the nucleus were modified.

8. Units in the rostral portion of the nucleus followed repetitive stimulation from the periphery at lower rates than those in the middle and caudal parts of the nucleus. Modified units followed repetitive stimulation at much lower rates than those not modified at every level.

9. On the basis of nuclear differences in distribution of cells by activating stimulus, corticofugal influences and the ability to follow repetitive stimulation in the periphery, it is suggested that a complex organization is present in n. gracilis; the rostral portion of the nucleus is functionally different from the middle and caudal portions, and the rostral portion may not contribute to the lemniscal system.

Significance to the Program of the Institute: This project emphasizes the functional complexity of this sensory relay nucleus and shows the important (Modulatory?) influence exerted upon it by higher centers. It also provides quantitative data for the small areas of body representation which make up the mosaic concerned with the perception of sensation.

Proposed Course of Project: Definitely completed. The paper has been presented at the Meeting of the American Electroencephalographic Society where it was selected as the most meritorious work by a junior investigator (see Part B)

Part B Yes [x] No [ ]

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Part B Honors, Awards and Publications:

This project was selected for the Hans Berger Award by the Women's Auxiliary of the American Electroencephalographic Society (Oct. 1964, Santa Fe, New Mexico).

Publications other than abstracts from this project:

Winter, D. L.: N. gracilis of cat. Functional organization and corticofugal effects. J. Neurophysiol. 28: 48-70, Jan. 1965.

Serial No: NDB(I)-63 EEG/CN 1001(c)
1. Electroencephalography and Clin. Neurophysiology
2. Clinical Neurophysiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Cortical inhibitory postsynaptic potentials and strychninization.

Principal Investigators: D. Pollen and C. Ajmone Marsan

Other Investigators: None

Cooperating Units: None

Man years:

Total:	0.7
Professional:	0.4
Other:	0.3

Project Description:

Objectives, Method, Significance, etc: Topical application of strychnine on the cortical surface results in an excessive, hypersynchronous neuronal firing which is considered characteristic of an epileptogenic focus. It is important to understand how the normal neuronal activity becomes "excessive" and how the firing of nervous impulses is converted into such "hypersynchronous" barrage. One currently accepted, though not yet crucially proven hypothesis, explains the effects of convulsant drugs by way of a selective impairment of inhibitory mechanisms.

Methods: Experiments are carried out in cat (acute, lightly anesthetized preparations). Standard intracellular recording technique is used in the motor cortex and pyramidal tract cells are identified by their antidromic activation. The behavior of postsynaptic potentials in such cells is studied before and after applications of strychnine.

Major Findings:
1. Various types of inhibitory postsynaptic potentials (evoked by stimulation of the cerebral peduncles and of a thalamic intralaminar nucleus or "spontaneously" occurring) have been studied in Betz cells and unidentified neurons in the precruciate cortex of the cat before and after topical strychninization.

2. In most cases, IPSPs (all types) were markedly depressed by the drug. After an IPSP had been depressed to zero, it was often replaced by a depolarizing potential which would gradually increase in amplitude as time elapsed. This replacement of IPSPs occurred without any change in the resting membrane potential.

3. The temporal and morphological characteristics of the depolarizing potential could be best analyzed in those instances of antidromic or recurrent inhibition. These potentials were found to occur at the same latencies and to have the same times to the peak amplitude, and the same durations as the IPSPs they replaced.

4. The possibility that these depolarizations represent unmasked, pre-existing, excitatory postsynaptic potentials was considered and discussed. Reasons are offered for considering it less likely than an alternative hypothesis whereby strychnine may actually convert an IPSP to a depolarizing potential. This could occur through a damaging effect on the subsynaptic membrane which would permit the passage of larger ions, principally sodium, during the action of the inhibitory transmitter. This mechanism of action would explain both the decrease and the "reversal" of IPSPs after strychninization.

5. The present hypothesis that strychnine may produce its effects by a "damaging" or modifying action on the subsynaptic membrane is an alternative to the hypothesis of Eccles that strychnine may sterically block the action of the inhibitory transmitter.

Significance to the Program of the Institute: This work extends that which has been carried out through the years in the field of experimental epilepsy. It is expected to throw some light on the pathophysiology of the epileptic condition.

Proposed Course of Project: Completed.

Part B included: Yes [x] No [ ]

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

Pollen, D. A. and Ajmone Marsan, C. Cortical inhibitory post-synaptic potentials and strychninization. J. Neurophysiol. 28: 342-358, March 1965.

Serial No: NDB(I)-65 EEG/OC 1224(c)
1. Electroencephalography and Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Epilepsy. A critical review of the literature for 1963-1964.

Principal Investigator: C. Ajmone Marsan

Other Investigator: K. Abraham

Cooperating Unit: None

Man Years:

Total:	0.5
Professional:	0.2
Other:	0.3

Project description:

Objectives, Method, Significance, etc: This is a review work which the principal investigator has been specifically required to prepare by the Editor of "Progress in Neurology and Psychiatry" (1965). This work has been completed and will form the chapter on "Epilepsy" in this volume. This chapter provides a brief summary of, or a brief critical comment on, each of more than nine hundred papers (published throughout the world in 1963-64) on different aspects of seizure disorders.

Part B included: Yes [x] No [ ]

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Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

Ajmone Marsan, C. and Abraham, K.: Epilepsy. In E. A. Spiegel (Ed.): Progress in Neurology and Psychiatry, New York, Grune & Stratton, 1965, chapter VIII, in press.

Serial No. NDB(I)-65 EEG/OC 1225(c)
1. Electroencephalography and Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Implanted electrode study of nocturnal sleep.

Principal Investigators: K. Abraham and F. Snyder

Other Investigator: C. Ajmone Marsan

Cooperating Unit: National Institute of Mental Health

Man Years (NDB-EEG only)

Total:	0.9
Professional:	0.3
Other:	0.6

Project Description:

Objectives, Method, Significance, etc: This study is concerned with the topographical distribution of sleep patterns in various cerebral structures in man during different stages of nocturnal sleep, with particular emphasis on the REM (rapid eye movement) phase.

Patient Material and Methods: Subjects included in this study are patients with extrapyramidal disorders or epilepsy, with multiple, chronically implanted electrodes in various cortical and deep structures. The electrical activity from these electrodes is recorded continuously throughout the night (7-8 hours), with simultaneous recording of muscle-tone and eye movements.

Major Findings: Up to date, overnight sleep records have been obtained on 14 patients; data are still to be analyzed.

Significance to the Program of the Institute: This study is a continuation of the work previously done, and partly still in progress in this Institute. Sleep patterns have not yet been clearly defined in various subcortical structures in man. It is hoped, that this study, although performed on a patient material with cerebral disease, will help to determine "normal" patterns of sleep in those structures examined and possibly provide some clue to the mechanism of nocturnal

seizures. Emphasis is particularly on the REM (paradoxical, low voltage fast or rhombencephalic) sleep which is suggestively related to dreaming and is currently a subject of intensive studies in several centers.

Proposed Course of the Project: All available and suitable patients will be studied with similar techniques, and the available data analyzed.

Part B included: Yes [ ] No [x]

Serial No: NDB(I)-65 EEG/CN 1226(c)
1. Electroencephalography and Clin. Neurophysiology
2. Clinical Neurophysiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Physical properties of cortical neurons.

Principal Investigator: H. D. Lux

Other Investigators: D. A. Pollen

Cooperating Unit: None

Man Years:

Total:	1.5
Professional:	0.9
Other:	0.6

Project Description:

Objectives: Within the last decade much work has focused on intracellular recording of synaptic drives upon neurons in the mammalian cortex. Whereas the complex cortical anatomy makes the task of untangling synaptic organization formidable enough, the problem is even further complicated when there is lack of knowledge of those physical properties (such as membrane resistance, capacitance, time constant, rectification properties and soma-dendritic conductance ratios) which set limits on the "output" function of a given cell in response to synaptic input.

These basic data must be provided not only for understanding the physiology of the "normal" cell but for considering the possible types of changes that may occur in the membrane properties of a cell after it has become "epileptic". Hence this study also attempts to see whether any of the above properties are altered in cortical neurons exhibiting excessive or "epileptic-like" discharges after strychninization.

Methods: Experiments were done in barbiturate anesthetized cats using glass micropipettes for intracellular penetration. When single electrodes were used for both stimulating and recording, a Wheatstone bridge was employed. "Double microelectrodes" have also been successfully inserted into some cortical neurons, and in these experiments

current was passed through one electrode while using the other for voltage recording.

Major Findings: The voltage changing curves for cortical neurons appear to deviate significantly from the form expected for simple exponential curves. It is postulated that such deviations are caused by variable degrees of dendritic dominance. The precise soma-dendritic conductance ratios according to the theory of Rall are being determined.

Regarding rectification properties, the current-voltage relationship does not depart much from a linear relationship in the normal physiologic range of membrane potential levels, and such small departures as have sometimes been found are still being evaluated for possible significance.

The above measurements as well as those for resistance and time constant, and the effects of strychninization upon the properties are currently being made.

Significance to the Program of the Institute: These data will allow a better understanding of the physical properties of "normal" cortical neurons. This in turn may allow us to further understand the functions of such cells, the similarities or differences with other central neurons previously studied, and will provide a base for seeing whether alterations in physical properties occur and are responsible for the excessive neuronal discharges of experimental epilepsy. This in turn may help in our understanding of the basic disorder in human epileptic states.

Proposed Course of Project: The experimental part of this work has been completed. Measurements are now being made on the large amount of available data. Calculations based upon these measurements have also begun and the preparation of the findings in publishable form should be ready in the next two-three months.

Part B included: Yes [ ] No [x]

Serial No: NDB(I)-65 EEG/CN 1227(c)
1. Electroencephalography and Clin. Neurophysiology
2. Clinical Neurophysiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Cortical neurons; conductance changes during IPSPs and an evaluation of anodal break and post-inhibitory rebound.

Principal Investigator: D. A. Pollen

Other Investigator: D. H. Lux

Cooperating Unit: None

Man Years

Total:	1.5
Professional:	0.9
Other:	0.6

Project Description:

Objectives: These experiments have been undertaken to determine the extent and duration of the membrane conductance changes during induced post-synaptic potentials (IPSPs) in neurons of the motor cortex of the cat; and to determine the respective contribution of both membrane conductance change and increase in membrane level to the inhibitory effect itself.

Secondly, it is at present an open question as to what extent post inhibitory membrane changes may contribute to the recycling mechanisms which seem to be involved in the genesis of the main cortical rhythms. Anodal break phenomena after passage of rectangular currents have been evaluated in different cells at different membrane levels. In order to ascertain the significance of rebound in a physiological situation these data are re-evaluated in view of the dampening effect of the prolonged inhibitory transmitter action.

Thirdly, the possibility is investigated that changes in the membrane properties of cortical neurons may be involved in the transition of a cell from normal to "epileptic" during the production of experimental "epileptiform" states as induced by topical application of strychnine. A related study (see previous project) deals with the possible changes in the physical properties of the resting membrane. This project deals with possible changes in the subsynaptic

membrane subserving the IPSP and with their possible alterations under the effect of strychnine.

Methods: Experiments were done in barbiturate anesthetized cats using glass micropipettes for intracellular penetration. When single electrodes were used for both stimulating and recording, a Wheatstone bridge was employed. "Double microelectrodes" have also been successfully inserted into some cortical neurons, and in these experiments current was passed through one electrode while using the other for voltage recording.

Major Findings: Both the conductance and voltage changes are appreciable during the peak of the IPSP. Conductance increases of over 50% have been seen during strong IPSPs and increases of at least 30% are not unusual. The conductance changes decrease but are still significant during the subsiding phase of the IPSP. This suggests a marked prolongation of either transmitter action or of its release.

Action potentials following the break of anodal currents occur in most cells if strong enough polarizing current is used. However, with membrane potential displacements in the usual physiologic range, the break response is marginal in most normal cells.

Conductance changes attending the decrease of IPSPs by strychnine are being measured at present.

Significance to the Program of the Institute: This and the associated work (see preceding project) provide data about the physical properties of the membrane both in the resting state and during inhibitory synaptic action. It is also hoped that the "epileptogenic" action of strychnine can be elucidated.

Proposed Course of Project: The experiments have been completed. Measurements and calculations are being made, the final results and conclusions should be available for publication within 2-3 months.

Part B included Yes ☐ No ☒

Serial No: NDB(I)-65 EEG/CN 1228(c)
1. Electroencephalography and Clin. Neurophysiology
2. Clinical Neurophysiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The effect of strychnine on ganglion cells.

Principal Investigator: H. D. Lux

Other Investigators: None

Cooperating Unit: None

Man Years

Total:	0.8
Professional:	0.2
Other:	0.6

Project Description:

Objectives: Parallel experiments to those described in the two preceding projects have been carried out in single cells of isolated leech ganglia, and are designed to supplement work and aims described in those two papers. Specifically this preparation had certain advantages not present in the mammalian cerebral cortex. Exact doses of drug could be applied. Any changes in action potential or synaptic potentials can be studied in a stable preparation for many hours.

Methods: Experiments to be carried out in the leech using glass micro-pipettes (single and double) for intracellular recording and for passage of current.

Major Findings: Plateau action potentials and decrease of height of the action potential (dose dependent) were observed after strychnine without significant change in resting potential or resting membrane resistivity. The synaptic potential analysis has not yet been completed.

Significance to the Program of the Institute: This study which parallels and complements those carried out in the central nervous system of mammals should increase our understanding of the basic properties of normal and "epileptic" neurons.

Proposed Course of Project: More experiments must be done before this project is completed.

Part B included: Yes ☐ No ☒

ANNUAL REPORT
July 1, 1964 through June 30, 1965
Laboratory of Neuroanatomical Sciences
National Institute of Neurological Diseases and Blindness

Alfred J. Coulombre, Chief

Introduction

During FY 65 the four Sections of the Laboratory of Neuroanatomical Sciences have undertaken a total of 30 projects, most of which have been brought to completion, and many of which have yielded results which form the basis of new projects which are now being undertaken. Every major segment of the nervous system has received attention. A balance has obtained in the attention given to the major sensory systems, the central nervous system, and the efferent pathways and neuromuscular systems. Investigation has been conducted at the gross, microscopic, and ultrastructural levels. It is especially true in the nervous system that a purview of organizations at all of these levels is essential to a complete understanding of the patterns of neural organization which form the morphological basis of function. In a program of this size the selection of the most suitable species for the study of a given phenomenon has quite naturally led to the use of vertebrate forms drawn from all five classes. The occasional use of the comparative approach has also tended to assure the use of a wide range of species in the work of the laboratory. During the past several years the interest in developmental phenomena such as cytogenesis, histogenesis and morphogenesis, which has always been a concern of this laboratory, has been slightly expanded and has come into balance with the other approaches used in the laboratory. The output of the laboratory during the past year can be summarized most succinctly by discussing each of the major segments of the nervous system in turn.

I. The sensory systems: Input to the nervous system occurs through a restricted number of sensory channels, each of which transduces qualitatively different type of information from the environment and encodes this information in such a way that it can be transmitted in the form of neural impulses to appropriate stations in the central nervous system. The Laboratory of Neuroanatomical Sciences devotes about half of its resources to an analysis of the structure and organization of the sensory input pathways. Each major pathway is under analysis at every level from the peripheral sense organ to the terminal connections which are made centrally. Structural analysis is carried out from the gross morphological level through the level of fine structure as revealed by the electron microscope. In many instances comparative analysis of the sensory systems is undertaken using representatives of each of the five vertebrate classes. Increasing attention has been focused on the sequence of individual steps which, during embryonic development, are responsible for the cytogenesis, histogenesis and morphogenesis of individual parts of each of the major sensory systems.

A. The auditory system: A battery of techniques (Golgi, Koelle, Marchi, Nauta-Gygax, Rasmussen, electron microscopy, etc.), coupled with experimental lesions placed at different levels of the auditory system of adult mammals (cat, chinchilla, primates) are revealing in increasing detail the complex interconnections that occur between the several levels of the auditory system, as well as between the auditory system and other portions of the central nervous system. During the past year the following specific findings have resulted: 1. A centrifugal pathway has been identified which connects the superior olive to the ventral cochlear nucleus. This pathway terminates in a plexiform synaptic arrangement on the cells of the ventral cochlear nucleus in conjunction with afferent caliciform endings of the cochlear nerve; 2. The discovery of a pathway connecting the nucleus of the trapezoid body with the homolateral superior olivary nucleus, coupled with the centrifugal pathway mentioned above, provides a missing link in the chain of neurons that interconnect the cochlear nuclei of both sides. Knowledge of this pathway offers an explanation for physiological data which until now has had no morphological basis; 3. The relative population of afferent and non-afferent synapses on the cells of the cochlear nuclei is different in different regions. The vast majority of the synapses terminating on the cells of the dorsal cochlear nucleus, as well as of the anterior one-third of the ventral cochlear nucleus, are of the non-afferent type. Most of the terminals on cells of the posterior two-thirds of the ventral cochlear nucleus are of the afferent type; 4. Changes in the cells of the cochlear nuclear complex are consistent with the notion that the degree of transneuronal atrophy is dependent upon the number or proportion of endings which have been destroyed by deaferentation; 5. Following deafferentation, the number of residual non-afferent type endings increases during the first post-operative month. This increase is possibly due to sprouting of non-afferent terminals, and raises interesting possibilities concerning dynamic balances which may exist between populations of different types of terminals on the surface of the same neuron. Recently investigations were begun with the electron microscope to explore fine structure, and fine structural changes, of the ventral cochlear nucleus following destruction of the spiral ganglion. 6. The medial geniculate body, which lies at a higher level in the auditory system, has a neuronal architecture in the cat which permits its division into three nuclei: medial nucleus; ventral nucleus with a laminar arrangement which may reflect a tonotopic organization of the fibers representing the cochlear input; dorsal nucleus which has a mixed input originating, in part, in the region of the midbrain medial to the brachium of the inferior colliculus, and, in part, from fibers that leave the optic tract. The dorsal nucleus may thus have an integrative function. This analysis has opened the way for a more meaningful physiological analysis of the medial geniculate body.

Experiments have been performed, and are now awaiting analysis, which will extend the types of findings previously made on lower mammalian forms to the primates.

The cytogenesis and histogenesis of the inner ear is under analysis in chick and mouse embryos. By injecting tritiated thymidine at known stages in development, and by subsequently analyzing the distribution of label in the cell nuclei of the vestibular and cochlear portions of the inner ear, the step

by step sequence by which these organs are formed is being established. Once base lines have been established it is planned to undertake similar analyses of a series of strains of mice, each of which bears a single mutant gene which interferes with one or another step in the developmental sequence. It should be possible to identify the time of onset and the nature of genetic defects, some of which are quite similar to congenital defects of the inner ear seen in man.

B. Olfactory system: Light and electron microscopic investigation of the olfactory mucosa revealed two types of cells: bipolar olfactory neurons, and supporting cells. The dendrites of the olfactory neurons have the following properties: 1. They are up to 200 micra in length; 2. their shafts contain single fibrils throughout most of their length; 3. vesicles lie between the ciliary membranes and the ciliary fibrils of the shaft; 4. divisions in the shaft which are bridged by double membranes occur with relatively high frequency; 5. the complex system of centrioles and basal bodies which exists at the dendritic apex of the bipolar cell suggests the possibility that the olfactory cilia are continually replaced rather than persisting as relatively permanent structures. In contrast the supporting cells have microvilli on their free borders, and contain granules which suggests that they are the source of mucuous material which is discharged onto the surface of the olfactory epithelium. These analyses have provided detailed knowledge of the fine structure of the probable primary olfactory transducers.

The analysis of the fine structure of the olfactory system was extended into the olfactory bulb of the rat with the following findings: 1. The major pathways in the superficial layers of the olfactory bulb were identified at the fine structural level; 2. synapses in the various layers were studied in detail. Dendrito-dendritic synapses were discovered. In addition, different synapses between the same two dendrites were shown to be morphologically polarized in opposite directions. These findings are of some importance since they reveal a new type of synaptic relationship between neurons, and which has already been useful in explaining electrophysiological data; 3. small periglomerular neurons were found to give rise to sheet-like processes which engulfed other periglomerular neurons.

C. Visual system: One program of the laboratory investigates the mechanisms which control the step by step development of the tissues of the vertebrate eye in such a way that they have sizes, shapes, positions, orientations and differentiated properties appropriate to optical function. It has been possible to identify many steps in a precisely timed series of interactions among the tissues of the eye, to specify which tissues are sources of specific influence, and which tissues are targets of influence; to determine the stages of development during which specific influences are mediated. As a consequence we have built a flow sheet which not only explains the orderly morphogenesis of this organ, but also provides a rational basis for understanding many of the defects that result from the interference with one or another step in its development.

A few of the specific findings of the past year follow: Last year work in this laboratory demonstrated a mechanism which operates to continuously control the orientation of the developing lens. The neural retina is the only tissue of the eye which has yet been shown to have a specific influence on the growth of the lens. The continued presence of the neural retina is essential for the normal growth and shaping of the lens. Work this year has revealed that this mechanism is not species, class or age specific, and that it consists of a water soluble, diffusible factor or factors arising from the neural retina. Lens tissue removed from the eye environment has only a limited capacity for sutonomous differentiation. Explantation of embryonic lens epithelium into defined or supplemented culture medium revealed that these cells are initially capable of partially differentiating into lens fibers in protein supplemented medium, that this ability is lost when cells are cultured in defined medium for 48 hours, and that lens epithelial cells which have been maintained in defined culture medium for 48 hours are still capable of forming lens fibers when returned to the environment of the eye but not when they are implanted in the coelom. In addition, it has been demonstrated that lens epithelial cells begin to divide in culture only after they have lost their ability to differentiate in supplemental media. These results are of importance since they have made available an in vitro test system which is capable of detecting the presence of the retina-lens factor. This opens the way for the isolation and identification of this factor or factors.

Further attention has been given to the interactions which occur between the neural and pigmented portions of the retina during development. Neural retina has been successfully regenerated in patches from the pigmented epithelium of the embryonic eye of the chick. This regeneration occurs when the neural retina is separated from the pigmented epithelium by a discrete distance. The pigmented epithelium will regenerate neural retina only under the influence of partially differentiated neural retina or otocyst epithelium. Other neural epithelia (e.g. optic tectum) will not support this regeneration. This study indicates the mechanism which assures continual apposition of the neural and pigmented portions of the retina during development (demonstrated by previous work in this laboratory) has, as one of its functions, the assurance that the pigmented epithelium will differentiate in the appropriate direction, and not form ectopic neural retina. In addition, the way is open for studies into the nature of the factor or factors arising from certain neural epithelia which will elicit regeneration of neural retina from the pigmented epithelium. A test system has been developed which is capable of detecting the presence of such a factor or factors.

Another specific tissue interaction has been demonstrated between the lens and the cornea. The presence of the lens is as necessary throughout the major portion of embryonic life to support the differentiation of the cornea. This finding makes it clear that the induction of the cornea by the lens is not mediated solely during a brief period early in development, but that this influence is necessary for the continued and orderly development of the cornea. An in vivo test system has been developed which may make possible the isolation of the factor or factors arising from the lens which influence the cornea.

Several clinical studies have suggested a higher incidence of lens cataracts among the offspring of diabetic mothers than occurs in the general population. We have accumulated experimental evidence that elevated concentrations of sugar (glucose, galactose, or xylose) in the embryonic blood stream at high enough levels and for a sufficiently long period during development, results in the production of lens cataracts in 100% of the cases. Upon returning blood sugar to normal levels, it was shown that damaged lens fibers underwent complete dissolution followed by removal of their products so that, after a period of time, the lesion was no longer visible, although the arrangement of fibers in the lens indicated the former location of the lesion. Only d-sugars were capable of bringing about these changes, and l-sugars proved ineffective in this respect. These findings emphasize the importance of maintaining careful control of the maternal blood sugar level during pregnancy. If experience in the chick embryo can be extrapolated to the human, elevated blood sugar levels would be much more harmful during the early stages than in the later stages of development.

A study of the development of the higher visual centers (optic tectum in the chick embryo) indicates that the retinotopic map in this neural epithelium develops in complete independence of the ingrowing optic nerve fibers. Thus, separate "maps" appear to develop at the retinal and tectal levels prior to the time at which the corresponding areas of both structures become interconnected by the axons of the ganglion cells of the retina. It is planned to expand these studies during the coming year to determine the sequence in which the neurons of the neural retina on the one hand, and the optic tectum on the other, become electrically active as embryonic development proceeds. In addition, we will determine the mitotic history, site of origin, and pathways of migration of the several neuronal components of the retina and optic tectum during the course of their development. Labelling with tritiated thymidine, followed by autoradiographic analysis, will be used in this investigation. Experiments have been designed to evaluate the extent to which the several levels of the visual system influence each other.

Attention has also been given to the neurological basis of color vision in fish. Removal of the anterior telencephalon did not result in a loss of color discrimination or generalization as tested in animals conditioned to respond to specific colors and brightnesses. Animals with posterior telencephalic lesions were able to discriminate the training stimuli but could not generalize either from the brightness or color characteristics of these stimuli.

II. Neuromuscular relationships: The principal output of the nervous system is to the several types of muscle that comprise the motor systems of the body. The Laboratory continues to explore the fine structure, the mechanisms of synaptic transmission, and the trophic interactions associated with the myoneural junctions of both striated and smooth muscle.

The relatively recent discovery of both granular and non-granular vesicles associated with nerve endings in smooth muscle has given rise to speculation concerning the role of these structures in synaptic and neuromuscular transmission. The finding of de Robertis and Iraldi that monoamine oxidase

inhibitors cause a profound increase in the numbers of granular vesicles in the sympathetic nerves of the rat pineal body could not be confirmed for similar nerve fibers in the vas deferens of the rat. Pargyline caused a rise in vas deferens norepinephrine of about 25%. While this was accompanied by an increase in the total number of vesicles per unit area of axon, there was no significant rise in the numbers of granular vesicles. A similar study in the nucleus dorsalis raphes likewise showed no increase in the numbers of granular vesicles in the adrenergic neurons in this nucleus following monoamine oxidase inhibition.

Administration of an antiserum to a nerve growth factor to rats and mice resulted in the absence of granular vesicles in nerves of the iris and submaxillary gland, but persistence at normal levels of granular vesicles in the vas deferens. This finding agrees with the relative uptakes of d-l-norepinephrine-$7H^3$ in these organs. Earlier hopes that the types of vesicles that cluster principally on the preterminal side of synaptic junctions might help to identify individual nerve fiber terminals on the basis of the neurohumor that characterized it have been partially realized. Proportions of granular and agranular vesicles may vary considerably in different locations and in different physiologic states. It remains to be demonstrated that changes in the population of vesicles provide consistent or sensitive index of physiologic states or of pharmacologic action.

Nerve fibers which terminate on muscle cells function not only to stimulate muscular contraction, but appear also to be necessary for maintaining specific types of metabolic activities in the muscle cells which they innervate. Such "trophic" interactions are under intensive study by the laboratory. Denervation was followed by a 50% decrease in cholinesterase in a period of 3 days. Partial denervation resulted in a loss of cholinesterase activity that was proportional to the fraction of the innervation that was interrupted. Reinnervation following denervation resulted in a slow recovery of cholinesterase activity in the muscle. Cholinesterase activity in hyperneuritized muscle was not greater than that of normally innervated muscle. Denervated muscle exhibits an increased blood flow, a decreased incorporation of C^{14}-leucine into protein, and an increased uptake of C^{14}-leucine into the free amino acid pool. Tenotomized muscle does not exhibit this increased uptake in the free amino acid fraction, but does show an equivalent decrease in incorporation into the protein fraction. These and similar studies are permitting us to come to grips at the molecular level with the nature of trophic interactions between nerves and muscle. It is becoming possible to specify which events at the chemical level are sensitive to the presence or absence of nerve, and which chemical events are relatively independent of innervation.

III. Central Nervous System: In addition to the primary sensory pathways discussed in the section on Sensory Systems, other portions of the central nervous system have received attention. The program of the laboratory is such that over a period of 3 or 4 years most of the major regions of the central nervous system come under active investigation.

This year a detailed analysis of the distribution of the thalamocortical fibers has been undertaken. These fibers distribute to all cortical layers

pre- and post-centrally, with heaviest concentration in layers IV and IIIb. In the visual and auditory areas of the cortex, the thalamic fibers distribute heavily in layers IV-IIIa with only occasional terminals in layer I. The increase in our knowledge of the neuronal cytoarchitecture of the cortex paves the way for understanding the manner in which this important layer functions, and provide a rational framework within which to understand problems of dysfunction.

A technical advance made during the past year should be of some help in the exploration of the cytoarchitecture of the brain. It is now possible to apply some neurological staining methods to thin sections cut from specimens of nervous tissue that have been processed in a manner suitable for electron microscopic studies. It should become possible to obtain from the same Golgi stained block one micron thick sections for light microscopic examination as well as ultra-thin sections for study with the electron microscope. This should make possible much more detailed reconstruction of the cytoarchitecture of portions of the nervous system than has hitherto been possible. It has already been possible to show that synaptic spines seen in Golgi stain material correspond to synaptic spines seen with the electron microscope.

Detailed studies have been completed of the normal fine structure of the locus coeruleus, nucleus dorsalis raphes, area ventralis tegmenti, and substantia nigra, with particular attention to the neuronal perikarya of these regions. Studies such as these are extending the information which is available from studies of the gross and light microscopic level to the level of organization just above the macromolecular level. Only by combining the information available from all of these levels of resolution will it be possible to completely define the structural organization of the nervous system which determines the way in which it marshalls and interrelates incoming information and patterns the outflowing impulses in such a way that appropriate motor responses result.

The closure of the neural folds early in development takes place during a relatively brief period of time. To determine the consequences of a failure of closure of the folds at different levels, closure was experimentally prevented at different levels along the neuraxis, and at different stages during closure of the folds. Among the findings the following are of interest: 1. The neural folds retain the ability to effect successful closure for only a brief period following the time in development at which they approach each other; 2. Failure of closure of the neural tissue at any level leads to overgrowth of the alar plate; 3. Interference with closure of the neural tube at any level greatly increases the frequency of lumbro-sacral spina-bifida; 4. Prevention of closure of the neural folds frustrates the accumulation of cerebro-spinal fluid in the ventricular spaces and spinal canal. Under these conditions the optic vesicle which evaginates from the diencephalic wall fails to expand completely, and either makes no contact with the surface ectoderm, or only tenuous contact. If no contact is made, anophthalmia results. If the extent of contact is subnormal, microphthalmia results. Thus, during that critical period in development immediately following the time of closure of the neural folds at which the optic vesicles are expanding, any interference with fold closure, or with the normal accumulation of cerebro-spinal fluid, can result in microphthalmia.

IV. Transfer of chemical substances across the boundaries of the brain: Nervous tissue is regionally selective of the chemical changes that occur across its boundaries and between its several compartments. Insight into the types of mechanisms which control the chemical environment of the brain can be gained by comparative studies.

During the past year it was demonstrated that Trypan-blue dye was excluded from the brain of the goldfish as it is from the mammal. As in the mammal, injury to the brain parenchyma permits localized penetration of the dye. The chloride space of the goldfish and rat brain are comparable. However, the thiocyanate space of the goldfish brain is 43%, whereas that of the rat brain is only 10%. By identifying such differences the way is opened for identifying the structural, functional, and chemical bases of such phenomena as the "blood-brain barrier" and the selective compartmentalization of chemical substances by which the brain of each species maintains an appropriate internal environment.

Another approach to this problem has utilized an electron opaque protein (ferritin) to assess the ability of the ependymal lining of the rat brain to take up large molecules. Electron microscopic examination of specimens from 10 to 210 minutes following intraventricular or intracisternal injection of ferritin into rats demonstrated that, although the bulk of the ferritin is taken up pinocytotically at the surface of the ependymal cells, appreciable amounts move between the cells. In another study distribution of ferritin within the brain was followed. The "submicroscopic" pericellular spaces of the brain were found to be diffusion channels and not merely potential spaces. Ferritin crossed the glial border of the cerebellar subarachnoid space and parenchymal capillaries by moving across glial cytoplasm. Once inside the parenchymal interspace, however, molecules are free to reach the plasmalemma of glial and neuronal cells without further crossing glial processes. The identification of pericellular spaces (about 200A wide) as true diffusion channels is a significant contribution to our knowledge of how substances are distributed within the brain. In order to properly understand the dynamics of action of substances ranging from pharmacologic agents to elevated body concentrations of such normal constituents as amino acids, the pathways and kinetics of their distribution in the several compartments of the nervous system must be understood. These basic studies are laying part of the ground work for this type of work.

The inner lining of the nervous system (ependyma) has also been studied with respect to its response to SV_{40} virus. In the presence of this agent ependymal cells form tumors. Where tumor cells are organized to form rosettes, their interrelationships are similar to the normal ependyma in which apical terminal bars are most commonly composed of a luminal zonula adhaerens followed immediately by a zonula occludens. Where the tumor cells occur in loose array their cytoplasm is highly evaginated, and few or no specialized junctions link the cells. The absence of junctions appear to be associated with those regions which are presumed to be invasive. It is of considerable interest that the presence or absence of cell to cell junctions of the type mentioned above may, at least in this instance, be correlated with a degree of invasiveness of an

induced tumor. While it would be premature to generalize this finding, attention will have to be given in future studies to the possibilities which are raised by it.

V. Degeneration and regeneration: Attention has been given to regeneration in the central nervous system in lower vertebrates. Regardless of the age in which total or subtotal ablation of the telencephalon is made, no restoration of the ablated parenchyma occurs. After total removal of the telencephalon the site of the lesion becomes covered with a sheet of columnar epithelium. After partial section of a hemisphere, cystic dilations (completely or partially lined with ciliated columnar epithelium) form at the site of the wound. This study has established that even in animals that are capable of regenerating fiber tracts in the spinal cord there are areas of the central nervous system that do not regenerate. The pattern of cellular reaction to central nervous system insults differs in mammals and fish.

Peripheral nerves are commonly isolated from the animal in electrophysiological investigations. A study was conducted to correlate changes in the electrical response of such isolated nerves (cat sciatic nerve, in this instance) to stimulation and to correlate this with the fine structural changes which took place under the conditions of isolation. Unmyelinated (C) fibers became unresponsive to stimuli after six hours in vitro. The myelinated (A) fibers failed after 8-3/4 hours of isolation. Nevertheless, the structure of these fibers appears normal for 12 and 24 hours following isolation respectively. Thus, no correlation could be found between the physiological and ultrastructural changes. Nerve fibers of the central nervous system (spinal cord) degenerate more rapidly than those of the peripheral nervous system when isolated in mineral oil. The electron microscope provides yet another tool for studying the sequence of changes which occurs, the morphology of nervous structures structures such as peripheral nerve during degeneration.

Regeneration of peripheral nerve has been studied in the sciatic nerve of rats and the vagosympathetic trunk of cats. By removing the nerves to a specially designed recording chamber at known intervals following crushing of the proximal portion of the nerve it was possible to follow, millimeter by millimeter, the progress of an electrically stimulated action potential as it coursed down the nerve. The crushed nerve fibers began to grow very slowly (0.3 to 0.5mm per day) and then accelerated exponentially to achieve a velocity of 3.0 mm per day after 18 days. At the level of the lesion conduction velocity reached 80% of normal by 28 days and remained unchanged thereafter. Ten to 15mm distal to the lesion the conduction velocity required 56 days to achieve 80% of normal. Spike height at any point along the nerve was but 25% of normal at 56 days postoperatively. The rate of regeneration in the vagus nerve of the cat is quite comparable to that of the rat sciatic nerve. This is the simplest and most reproducible technique that has yet been devised for studying the rate of regeneration of peripheral nerves. It can be used for pharmacological studies and correlative biochemical investigations. It is planned to utilize this technique for studying the effects of such substances as the drugs of malanonitrile group, colchicine and other agents upon the rate of nerve regeneration. In addition, biochemical studies are planned

on the sequence of changes in protein synthesis in spinal ganglion cells during the process of regeneration, with and without treatment with pharmacologic agents that influence nerve regeneration.

VI. Future of the program: In order to accomplish its mission the laboratory has evolved and maintained a highly diversified folio of projects dealing with the structure and function of the nervous system. While the spectrum of projects under study shifts from year to year in order to meet changing needs, and to take advantage of new opportunities, each of the major components of the nervous system has received attention every year. The laboratory has not committed itself to any one technique or group of techniques, nor to any one conceptual framework. Diversity has been achieved by structuring the staff in such a way that a wide range of approaches are represented in the environment, and at the same time encouraging individual investigators to pursue their problems in depth. The present position and probable future course of our program can best be brought into focus by listing the major areas which are currently under attack.

1. An important goal of the laboratory since its inception is to analyze the structural organization of the nervous system. The auditory, vestibular and visual systems are under intensive gross and microscopic analysis at the present time. The results of these investigations provide a basis for understanding function and dysfunction. The ongoing program concerned with neuronal interconnections among the different levels of the auditory and vestibular systems is a notable example of this type of undertaking. At the present time it is the only major effort in the intramural programs of NIH which deals with the structure and function of the auditory pathways.

2. The laboratory has made increasingly meaningful contributions to our knowledge of the fine structure of the nervous system as revealed by electron microscopy. In the past the cytology of most of the major cell types of the nervous system has been intensively investigated. All but exhaustive inventories of cell organelles in glia, motor neurons, neurosecretory cells, ependyma, etc. have resulted from these studies. More recently the types of junctions that occur between cells (including synapses and myoneural junctions) have been analyzed. The structure of the junctions between nerve fibers and smooth muscle cells has received particular attention. Work on the fine structure of the nervous system will continue to be an important part of our program.

3. Increasing attention has been given to the trophic interactions which occur between parts of the nervous system, and between the nervous system and the periphery. For example, the molecular mechanisms underlying the various types of muscle atrophy (atrophy of denervation, atrophy of disuse, etc.) is being analyzed by studying the control of the protein metabolism of skeletal muscle which is exerted by peripheral nerve fibers. In addition very satisfactory progress has been made in identifying specific interactions among the tissues of the eye and the ear. By developing assay systems which are sensitive to factors arising in one tissue which affect course of differentiation or morphogenesis of adjoining tissues, a way has been opened for the possible isolation of a new class of substances (morphogens). The isolation of such substances would greatly increase our ability to control the regeneration of

specific parts of organs. One example of this is the demonstration that there is a water soluble, diffusible influence arising from the neural retina which controls the differentiation of cells in the lens.

4. Intensive studies of the morphogenesis of the eye and the ear are making available detailed flow sheets which describe step by step the differentiation and morphogenesis of these organs. Such flow sheets identify the critical periods during development in which teratogenic influences (pharmacologic agents, genetic aberrations, infections, etc.) will produce specific congenital abnormalities. It has been possible to show that elevated blood sugar is an etiologic factor in the production of congenital lens cataract. Also, interference with the closure of the neural folds during a brief but critical period in the development of the nervous system can lead to microphthalmia. A whole range of auditory and vestibular defects can be predicted on the basis of the specific stage at which an untoward influence is brought to bear. It is anticipated that this work on normal and abnormal morphogenesis will continue. Part of this program will include the use of a wide range of pharmacological agents in order to assess their effects upon the developing sensory and nervous system, using techniques developed in this laboratory.

5. The laboratory has given attention in the past to the regeneration of the peripheral nerve, especially with respect to neuromuscular specificity. In the immediate past we have developed techniques which make it possible to follow the rate of regeneration in peripheral nerve with some accuracy. It is planned to apply these methods to an assessment of substances and conditions which may favor more speedy and more satisfactory regeneration of peripheral nerves. In addition, attention will be given to the role of protein synthetic systems located in the nerve cell body which may have an important bearing on the rate and quality of regeneration.

6. The development of new techniques, while not a specific objective of our program, is a by-product of the work done in this laboratory. In the past we have contributed importantly to techniques of fixation and staining of material prepared for histologic and electron microscopic examination.

Among other techniques currently being developed is one which may permit routine serial thin sectioning of specimens prepared for electron microscopy.

Serial No. NDB(I) - 60 LNS/FN 712
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The ascending and descending auditory connections in primates.

Principal Investigators: Grant L. Rasmussen

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 mo. period)
Total: 1.4
Professional: 0.2
Other: 1.2

Project Description:

Objectives: To extend previous studies on lower mammalian subjects to primates that possess anatomical features more similar to those of man.

Methods Employed: Experimental neuroanatomical methods are employed following placement of lesions in various auditory nuclei and fiber pathways including the auditory cortex. Resultant fiber and synapse degeneration is observed with the aid of the Marchi, Nauta-Gygax and the the Rasmussen synapse methods.

Major findings: Insufficient number of experiments have been completed on the primate to report major findings at this time.

Significance: The anatomy of the primate auditory system has not been studied sufficiently with experimental methods to ascertain any major findings.

Proposed Course of Project: To carry out more experiments in higher auditory centers after completing study of levels caudal to inferior colliculus. Thus far, lesions have been made in the lower auditory levels of seventeen cynomolgus monkeys and the brain histology completed.

Part B Included: No

Serial No. NDB(I)-60LNS/FN 713
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: A study of the auditory vestibular afferent and efferent systems including the receptors.

Principal Investigator: Grant L. Rasmussen

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 mo. period)
Total: 2.5
Professional: 0.7
Other 1.8

Project Description:

Objectives: To continue to explore and reveal unknown anatomical neuronal connections of the afferent and efferent divisions of the auditory system; to gain more information about the anatomical and functional interrelationships of these two systems. The immediate problem under study is to establish at the synaptic level the interconnections existing between the cochlear nuclei, superior olivary complex, the nuclei of the lateral lemniscus and the inferior colliculus as well as the auditory reflex connections with certain motor nuclei and the reticular formation.

Methods Employed: The newer and more effective techniques are chiefly relied upon for demonstration of axonal, preterminal and terminal degeneration. Also the Golgi and the histochemical method of Koelle, which we have modified to advantage, are heavily relied upon to furnish information not otherwise obtainable. Since the efferents of the cochlear nerves exhibit a much higher concentration of acetylcholinesterase than the afferents it is possible to differentiate in histological preparations the two functionally different types of fibers.

The experimental anatomic studies dealing with synaptic connections of the ascending and descending auditory system has been extended and new information gained. Some significant findings as follows were reported at the International Symposium on Sensorineural Hearing Processes and Disorders this year and will appear as a chapter in a book to be published by Little, Brown & Company.

(a) The opinion, (Rasmussen, 1964) that the descending or feedback fibers and their synapses are characterized by high AChE content has been strengthened by additional findings. The centrifugally conducting fibers from the superior olive to the ventral cochlear nucleus are traceable with the histochemical method of Koelle to their synaptic termination in the ventral cochlear nucleus where the fibers end as a plexiform synaptic arrangement on cells along with the afferent calcyciform endings of the cochlear nerve. This chemical difference holds true for a recently disclosed connection between the nucleus of the trapezoid body and the homolateral superior olivary nucleus described below.

(b) This heretofore unknown connection was demonstrated in cat and chinchilla, with axonal degeneration techniques. This finding is of particular importance in that it provides the missing link in the chain of neurons that interconnect the cochlear nuclei of both sides. Physiological experiments indicate that a crossed connection should exist since the stimulation of one ear causes marked changes in neural activity of the opposite cochlea nucleus even when it has been deprived of cochlear nerve input.

(c) A study of the relative population of afferent and non-afferent synapses in the cochlear nucleus has received the most attention this year. After elimination of the afferent endings by destruction of the spiral ganglion in cat and chinchilla the remaining non-degenerate efferent type synapses were always observed in impressive numbers. The relative proportion of the two types of synapses varied in the different subdivisions of the cochlear nucleus. The vast majority of synapses of the dorsal cochlear nucleus are the non-afferent type while those of the ventral nucleus are in general predominantly of the afferent type, with one exception, namely the rostral third of the anterior ventral nucleus. This study offers for the first time a quantitative evaluation

of the relative population of the two types of synaptic connections in the cochlear nucleus and points up the great complexity of this center which evidently is capable of processing auditory information for transmission to higher auditory levels. In a time course study of 1-6 months the synapses of the cells in the different subdivisions of the deafferentiated cochlear nucleus shrunk to different degrees. The posterior ventral nucleus which possessed the greatest number of cochlear afferent endings atrophied to a greater extent than did cells of the anterior ventral nucleus which possessed a greater proportion of non-afferent type endings. It is concluded from these experiments that the degree of transneuronal atrophy is dependent on the number of functional synapses. Another significant finding in this study is that the residual non-afferent type endings appear to increase in number after the first post-operative month. This increase in population is thought to be due to sprouting of the non-afferent terminals.

Significance: Knowledge of the neuronal relationships existing at the synaptic level between the afferent and efferent systems is basic to an understanding of the neuromechanism of hearing. Such information is essential for a foundation upon which to design physiological experiments for testing the functional significance particularly of the descending conduction system.

Proposed Course of Project: (1) The study of the efferent innervation of the vestibular receptors with Dr. C. Smith, Department of Otolaryngology of St. Louis will be continued. (2) Anatomical studies of the ascending and descending auditory systems will be continued and a collaborative EM study of the deafferentiated cochlea nuclei will be continued with Dr. Reese of this laboratory. (3) A collaborative anatomico-physiological study will commence in June with Dr. Desmedt in his laboratory in Brussels, Belgium, concerning the functional role of descending auditory system fibers.

Part B Included: No

Serial No. NDB(I)-60 LNS/FN 714
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Study of the afferent extrinsic and intrinsic neuronal connections of the medial geniculate body.

Principal Investigator: Donald Kent Morest

Other Investigators: Grant L. Rasmussen

Cooperating Units: None

Man Years (computed for the 12 mo. period)
Total: 0.1
Professional: 0.1
Other: 0.0

Project Description:

Objectives: (1) To disclose the neuronal architecture and the intrinsic interneuronal connections in the medial geniculate body itself by means of the Golgi methods.

(2) To locate the origin and course of the afferent projections of the brain stem to the medial geniculate body that are located outside the well known auditory pathways.

Methods Employed: Suitable Golgi methods will be used to elucidate the cellular morphology and intranuclear connections of the medial geniculate. Modified Bielschowsky methods for demonstration of axonal and preterminal degeneration subsequent to strategically placed lesions in the brain stem.

Major Findings: This study has disclosed much needed detailed fundamental cytomorphological information about the medial geniculate body. An analysis of the basic neuronal architecture, extrinsic and intrinsic connections in the cat as studied by different methods of approach is the subject of three manuscripts prepared for publication.

Significance: This study establishes an anatomical basis for future psycho-physiological studies on the functional role played by different subdivisions of the medial geniculate body and particularly to test physiologically the function of the dorsal nucleus of the medial geniculate body.

Proposed Course of the Project: As a result of these investigations two papers have been published and the third, entitled "The lateral tegmental system" has been accepted by the British Journal of Anatomy.

The objectives of the project have been achieved and the project is now completed.

Part B Included: Yes

Part B Honors, Awards, and Publications

Publications

Morest, D. Kent: The neuronal architecture of the medial geniculate body of the cat. J. Anat., London, 98: 611-630, December, 1964.

Morest, D. Kent: The laminar structure of the medial geniculate body of the cat. J. Anat., London, 99: 143-160, February, 1965.

Serial No. NDB(I)-60 LNS/EN 711
1. Neuroanatomical Sciences
2. Section on Experimental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Regeneration in the peripheral nervous system

Principal Investigator: Lloyd Guth

Other Investigators: Stanley Jacobson

Cooperating Units: None

Man Years (computed for the 12 month period)

Total:	.7
Professional:	.6
Other:	.1

Project Description:

Objectives: To study the factors influencing the rate of regeneration in the peripheral nervous system.

Methods Employed: At various times after crushing the sciatic nerve of rats or the vagosympathetic trunk of cats, the nerve was removed and placed in a specially designed recording chamber. The proximal end of the nerve was stimulated and the resultant action potential recorded mm by mm down the nerve. The distance at which the last action potential was recorded is an estimate of the distance that the fibers regenerated. Changes in spike height and conduction velocity were computed at 5 mm intervals along the nerves.

Major Findings: The technique has provided a sensitive method for evaluating the rate of nerve regeneration as well as the electrical characteristics of regenerating nerve fibers. A mathematical equation describing the growth of the nerve fibers was fitted to the data, and from the first derivative of this equation the instantaneous velocity of the fiber outgrowth was calculated day by day. The crushed nerve fibers begin growth very slowly (0.3 to 0.5 mm/day) and then accelerate exponentially

Part A Project Description (cont'd)

to achieve a velocity of 3.0 mm/day after 18 days. At the level of the lesion conduction velocity reached 80% of normal by 28 days and remained unchanged thereafter. Ten to 15 mm distal to the lesion the conduction velocity required 56 days to achieve 80% of normal. Spike height at any point along the nerve was but 25% of normal at 56 days postoperatively. The rate of regeneration of long peripheral nerves (vagus of the cat) is quite comparable to that of the rat's sciatic nerve. Measurement of the rate of return of pupillary size and ear temperature after sympathetic nerve lesions in the cat indicate that once the regenerating nerve fibers reach the postganglionic cells of the superior cervical sympathetic ganglion, very little additional time is required to establish a functional synapse with the pupillary postganglion cells, but several weeks are required for maturation of a functional synapse upon the vascular postganglionic cells.

Significance: This is the simplest and most reproducible technique yet devised for studying the rate of nerve regeneration. It can be used for pharmacological studies and correlative biochemical investigations such as those indicated in the following section.

Proposed Course of Project: The effect of drugs which increase protein synthesis in nerve cells (drugs of the malanonitrile group) will be studied for a possible enhancing effect on the rate of nerve regeneration. A drug which inhibits Schwann cell proliferation in transected nerves (colchicine) will be administered to evaluate the role of Schwann cell as a requirement for adequate nerve regeneration. Biochemical studies on the sequence of changes in protein synthesis in spinal ganglion cells (after nerve transection) will be correlated with the sequence of changes in rate of regeneration of their nerve fibers.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Jacobson, S. and Guth, L.: An electrophysiological study of the early stages of peripheral nerve regeneration. Exper. Neurol. 11: 48-60, Jan. 1965.

Honors and awards relating to this project: None

Serial No. NDB(I)-62 LNS/EN 928
1. Neuroanatomical Sciences
2. Section on Experimental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The neurological basis of color vision in the fish

Principal Investigator: Jerald J. Bernstein

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)

Total:	.5
Professional:	.3
Other:	.2

Project Description:

Objectives: To determine the neural pathways of color vision in the fish.

Methods Employed: The role of the nervous centers in the fish brain responsible for color visual integration is being studied. After extirpation of various portions of the telencephalon, fish were trained to make a color visual discrimination. A conditioned heart rate deceleration was the criterion for learning. Animals were tested to ascertain the ability to respond to the original colors (discrimination) and to respond to various shades of these colors (generalization). It has been found that normal fish react to the color of the stimuli regardless of shade (generalization) whereas immediately after extirpation of the telencephalon they do not. Animals were tested after extirpation of the following portions of the telencephalon: a) anterior portion; b) posterior portion; c) complete removal.

Major Findings: Removal of the anterior telencephalon did not result in a loss of color discrimination or generalization. Animals with posterior telencephalic

Part A Project Description (cont'd)

lesions were able to discriminate the training stimuli but could not generalize either from the brightness or color characteristics of these stimuli.

Significance: The area of the telencephalon involved with color vision in the fish has been determined and the cortical region mediating this function has been demonstrated. The fact that color generalization can be abolished without loss in discrimination by removal of the posterior telencephalon indicates that lower centers are involved in color vision and that there may be an anatomical seperation of the functions of color visual discrimination and generalization in the goldfish.

Proposed Course of Project: The project will be discontinued in 1965.

Part B included: No

Serial No. NDB(I)-62 LNS/EN 929
1. Neuroanatomical Sciences
2. Section on Experimental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Central nervous system regeneration in lower vertebrates

Principal Investigator: Jerald J. Bernstein

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)

Total:	.6
Professional:	.3
Other:	.3

Project Description:

Objectives: By comparing the regenerative capacity of different portions of the central nervous system of lower vertebrates we hope to clarify the mechanism of central nervous system regeneration. We hope to ascertain whether regeneration in lower vertebrates involves the formation of new nerve cells in addition to the outgrowth of severed nerve fibers.

Methods Employed: Various portions of the brain of the goldfish were removed. The ability of the operated animal to reconstitute the extirpated structure was determined histologically. The ability of the goldfish to repair different types of lesions (e.g. ablations versus simple transection) will be evaluated to determine the limitations of primary and secondary repair of CNS and tissue in this species.

Major Findings: Regardless of age at the time of operation removal of any mass of the telencephalon of the goldfish does not result in a restoration of the ablated parenchyma. After total removal of the telencephalon, the site of the lesion becomes covered with a sheet of

Part A Project Description (cont'd)

columnar epithelium. After partial section of a hemisphere cystic dilations form at the site of the wound. These cysts are completely or partially lined with ciliated columnar epithelium.

Significance: This study has established that even in animals that are capable of spinal cord regeneration there are areas of the central nervous system that do not regenerate. The pattern of cellular reaction to central nervous system insult differs in mammals and fish.

Proposed Course of Project: Because of Dr. Bernstein's resignation from the NIH, this project will be discontinued in 1965.

Part B included: No

Serial No. NDB(I)-63 LNS/EN 1054
1. Neuroanatomical Sciences
2. Section on Experimental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: "Trophic" functions of the peripheral nervous system

Principal Investigator: Lloyd Guth

Other Investigators: R. Wayne Albers

Cooperating Units: Laboratory of Neurochemistry

Man Years (computed for the 12 month period)

Total:	1.7
Professional:	.7
Other:	1.0

Project Description:

Objectives: To investigate the nature and mechanism by which the peripheral nerve influences the metabolism of its end-organ.

Methods Employed: 1. The changes in cholinesterase activity of muscle was determined after following alterations of the innervation: total denervation, partial denervation, reinnervation, hyperneuritization.
2. Radioactive tracer methods have been employed to study the blood flow and the rate of incorporation of amino acids into free amino acid and protein fractions of denervated and tenotomized muscle.

Major Findings: 1. Following total denervation there is a 50% decrease in cholinesterase in 3 days and the activity remains at that level for the ensuing several months. Partial denervation results in a loss of ChE that is proportional to the fraction of the innervation that was interrupted. Following reinnervation (after total denervation) the sole-plate ChE and non-sole-plate ChE is restored very slowly; it reaches nearly normal levels only after 6 weeks. The ChE activity of hyperneuritizied

Part A Project Description (cont'd)

muscle is not greater than that of normally-innervated muscle. 2. Denervated muscle exhibits an increased blood flow, a decreased incorporation of C^{14}-leucine into protein, and an increased uptake of C^{14}-leucine into the free amino acid pool. Tenotomized muscle does not exhibit this increased uptake in the free amino acid fraction, but does show an equivalent decrease in incorporation into the protein fraction.

Significance: Although the nerve exerts some control over the cholinesterase metabolism of muscle, the maximum enzymatic activity that can be achieved is apparently an intrinsic property of the muscle. Furthermore, the several weeks required for a reinnervated muscle to resynthesize normal levels of cholinesterase indicates that the neural factor does not act at the rate limiting step in the synthesis of the enzyme. The observation that denervation alters the free amino acid pool of muscle and tenotomy does not raise the possibility that the nerve regulates protein synthesis not by directly supplying the muscle with a necessary metabolite, but by altering the properties of the muscle membrane in such a way as to facilitate transport of metabolites into the cell.

Proposed Course of Project: New techniques will be developed to enable us to study (quantitatively and individually) the major steps in muscle protein synthesis in vitro. This project will constitute the major fraction of the coming year's work.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Guth, L., Albers, R. W., and Brown, W. C.: Quantitative changes in cholinesterase activity of denervated muscle fibers and sole plates. Exper. Neurol. 10: 236-250, Sept. 1964.

Guth, L. and Brown, W. C.: The sequence of changes in cholinesterase activity during reinnervation of muscle. Exper. Neurol. Aug. 1965 (in press).

Honors and awards relating to this project: None

Serial No. NDB(I)-63 LNS/EN 1055
1. Neuroanatomical Sciences
2. Section on Experimental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Mechanisms of chemical uptake in the central nervous system of lower vertebrates

Principal Investigator: Jerald J. Bernstein

Other Investigators: Eugene Streicher

Cooperating Units: Section on Clinical Neuropathology
Surgical Neurology Branch

Man Years (computed for the 12 month period)

Total:	.5
Professional:	.4
Other:	.1

Project Description:

Objectives: By comparing the blood-brain-barrier and the cerebral chloride and thiocyanate spaces of fish and mammals we hope to arrive at generalizations concerning the phylogenetic evolution of these physiological mechanisms.

Methods Employed: Trypan blue or sodium thiocyanate was introduced either directly into the water of the aquarium, or was injected intraperitoneally into the goldfish. The distribution of the trypan blue was studied histologically, and the distribution of the exogenous thiocyanate (as well as of endogenous chloride) was determined chemically for brain, muscle and plasma.

Major Findings: 1. Trypan blue dye was excluded from the brain of the goldfish as it is from the mammal. As in the mammal, injury to the brain parenchyma permits localized penetration of the dye. 2. The chloride space of goldfish and rat brain are quite comparable (33% and 30% respectively). 3. The thiocyanate space of the goldfish brain is 43% whereas that of the rat brain is

Part A Project Description (cont'd)

only 10%.

Significance: Phylogenetic similarities as well as differences between the blood-brain-barrier mechanisms have been observed. The mechanism by which dyes and anions are excluded from the brain is not fully understood but probably results from the interaction of a variety of anatomical, physiological and biochemical factors (e.g. glia and endothelial cell relationships, permeability of neurones and glia, and active transport mechanisms). Correlation of differences in barrier phenomena of fish and mammal with differences in structure, function, and chemistry of the tissues involved may ultimately clarify the complex blood-brain-barrier mechanisms in both species.

Proposed Course of Project: Because of Dr. Bernstein's resignation from the NIH we will discontinue this project for the time being.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Bernstein, Jerald J. and Eugene Streicher. The blood-brain barrier of fish. Exper. Neurol. 11 (in press).

Honors and awards relating to this project: None

Serial No. NDB(I)-63 LNS/EN 1056
1. Neuroanatomical Sciences
2. Section on Experimental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Intracortical distribution of thalamocortical fibers

Principal Investigator: Stanley Jacobson

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)

Total:	.2
Professional:	.1
Other:	.1

Project Description:

Objectives: To determine: 1) the laminar distribution of thalamocortical fibers in precentral, postcentral, visual and auditory areas of the squirrel monkey; 2) whether their distribution varies according to cortical region.

Methods Employed: Lesions were placed stereotaxically in the thalamus. Following a suitable postoperative survival the animals were sacrificed and their brains stained with Nauta's method for degenerating axons.

Major Findings: In precentral and postcentral groups the thalamocortical fibers distribute in all cortical layers with heaviest degeneration seen in layers IV and IIIb; in visual and auditory areas the thalamic fibers distribute heavily in layers VI-IIIa with only occasional terminals in layer I.

Significance: The difference noted in intracortical distribution of thalamic fibers indicates that the layers in motor and sensory cortex have more generalized intracortical and extracortical connections than those of other cortical areas. The layers of auditory and visual cortex show a greater degree of specialization. Layers

Part A Project Description (cont'd)

I to IIIa connect only intracortically and layers IIIb to VI exhibit both intracortical and extracortical connections.

Proposed Course of Project: Dr. Jacobson is leaving NIH in 1965 and this project will be continued at Northwestern University, Chicago, Ill.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Jacobson, S.: Intralaminar, interlaminar, callosal and thalamocortical connections in frontal and parietal areas of the albino rat cerebral cortex. J. Comp. Neurol. 124: 131-145, Feb. 1965

Honors and awards relating to this project: None

Serial No. NDB(I)-63 LNS/EN 1057
1. Neuroanatomical Sciences
2. Section on Experimental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The application of neurological stains to thin sections of central nervous tissue and the ultrastructure of nervous tissue

Principal Investigator: Stanley Jacobson

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)

Total:	.8
Professional:	.6
Other	.2

Project Description:

Objectives: 1) To extend neurological staining to thin sections of CNS, 2) to evaluate CNS structure with the light microscope on the basis of these methods, 3) to examine CNS tissue with electron microscope.

Methods Employed: 1) One micron sections were prepared from brains fixed in buffered gluteraldehyde and stained by the Nauta silver impregnation and the Nissl stain. 2) Slices of rat cortex and thalamus were impregnated by the Golgi method, then were embedded in methacrylate, sectioned at 1μ, and examined with light microscope. 3) Isolated sciatic nerve and spinal cord were examined physiologically and were then fixed in buffered osmium, embedded in araldite, sectioned, and examined with the electron microscope.

Major Findings: 1) The silver stains show intraneuronal organelles and mitochondria in synaptic vesicles and outlines the limiting membrane of the neuron. 2) The Golgi method on 1μ sections demonstrates that the synaptic spines seen in Golgi stained material correspond to synaptic spines seen with the electron microscope. The

Part A Project Description (cont'd)

Golgi neuron stain encrusts the cell with a superficial deposit. 3) The unmyelinated fibers (C fibers) become unresponsive to stimuli after 6 hours in vitro. The myelinated fibers (A fibers) fail after 8 3/4 hours of isolation. Since the structure of these fibers appear normal for 12 and 24 hours respectively, it is clear that there is no correlation between the physiological and ultrastructural changes. Nerve fibers of the central nervous system degenerate more rapidly than those of the PNS when isolated in mineral oil.

Significance: 1) The Nauta silver method applied to 1μ sections clearly demonstrated organelles which are poorly differentiated in thicker sections. 2) It has been presumed that the spines were an artifact of the Golgi stain; it is now clear that the Golgi stain encrusts the dendritic spines. 3) Peripheral nerves are commonly isolated from the animal in electrophysiological studies, but the effects of isolation on these nerves had not been previously determined. It is remarkable that peripheral nerves isolated from any nutriment in an anoxic media can maintain their structural integrity with minimal changes for as long as 24 hours.

Proposed Course of Project: Dr. Jacobson is leaving NIH and this project will be continued at Northwestern University, Chicago, Illinois.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Jacobson, Stanley Electrophysiological and Ultrastructural changes in Isolated Sciatic Nerves. Exper. Neurol. September 1965 (In press).

Honors and Awards relating to this project: None

Serial No. NDB(I)-61 LNS/NC 808
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The innervation of smooth muscle

Principal Investigator: Keith C. Richardson

Other Investigators: I. A. Michaelson, F. Sjöqvist, P. Taylor and E. Titus

Cooperating Units: Laboratory of Chemical Pharmacology, National Heart Institute

Man Years (computed for the 12 month period)

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives: To study the ultrastructure of autonomic nerve endings on smooth muscle in collaboration with chemical and pharmacological investigations.

Methods Employed: Standard electronmicroscopical techniques of osmium tetroxide fixation and epoxy resin imbedding.

Major Findings: The work quoted in the previous report of extensive serial sectioning for electronmicroscopy, for which a new technique has been devised, has had to be abandoned because both electronmicroscopes at our disposal lack double condensers which are necessary to protect the carbon-coated membranes bearing the serial sections from disruption. We are, therefore, limited at present to the examination of not more than 25 successive sections which is too few to establish further details of the nerve-muscle relationships.

Recent work on the homogenization and differential centrifugation in sucrose gradients of the whole heart (I. A. Michaelson) has been successful in isolating a

Part A Project Description (cont'd)

fraction rich in norepinephrine which consists electronmicroscopically of small and large vesicles, many of which closely resemble the nongranular vesicles of the nerve endings. There are, however, very few granular vesicles in these preparations. We have, therefore, paid further attention to the conditions of fixation, both of the homogenates and the intact tissues, to determine whether the dense cores of the granular vesicles may be lost with inappropriate fixation. Variations in pH. and in the type of buffer used in the fixative appear not to influence the granules. One tentative explanation of the results would be that the granular vesicle population is diluted, not only with the nongranular vesicles already in the adrenergic endings, but also with similar vesicles in cholinergic endings.

The concept that the granular vesicle may be a long-term storage entity in nerves which is variable and less important than the more numerous nongranular vesicles, has been tested by examining the rat vas deferens at the pre- and post-natal stages. It has been found that the autonomic axons are present within the vas deferens wall before the smooth muscle fibres are differentiated from mesenchyme, and that they contain granular vesicles probably in higher concentration relative to the nongranular type, than adult nerves.

Antiserum to a nerve growth factor causes profound atrophy of the paravertebral sympathetic ganglia (Levi-Montalcini, Cohen). In collaboration with the Laboratory of Chemical Pharmacology, National Heart Institute, tissues from immunosympathectomized mice and rats have been examined electronmicroscopically. The iris, submaxillary gland and vas deferens were still found to be richly innervated, but there was a lack of granular vesicles in all nerves except in the vas deferens. This finding agrees with the relative uptakes of d,l-Norepinephrine-7H^3 in these organs.

Significance: This work adds further information to the current controversies concerning the nature of smooth muscle-nerve relationships and provides a structural background to chemical and pharmacological investigations of the Laboratory of Chemical Pharmacology.

Part A Project Description (cont'd)

Proposed Course of Project: Two manuscripts are in course of preparation.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Michaelson, I. A., Richardson, K. C., Snyder, S. N. and Titus, E. O.: The separation of catecholamine storage vesicles from rat heart. Life Sciences, 3: 971-978, Sept. 1964.

Honors and awards relating to this project: None

Serial No. NDB(I)-61 LNS/NC 809
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The uptake of ferritin by the ependyma of the rat brain

Principal Investigator: Milton W. Brightman

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 0.2
Professional: 0.2
Other: 0.0

Project Description:

Objectives: To study the participation of the ependyma in the passage of protein molecules from ventricle to brain parenchyma.

Methods Employed: From 10 to 210 minutes following the intraventricular or intracisternal injection of ferritin into rats, the ependyma is fixed in osmium solutions and examined electronmicroscopically.

Major Findings: Although the bulk of the ferritin which moves across the ependyma is incorporated pinocytotically, appreciable amounts move intercellularly.

Significance: The ependymal epithelium is exceptional in that its luminal junction is not the usual "closed" intercellular fusion, but rather an "open" adhering fascia. Ferritin molecules gain access to the extracellular space by first passing along the interspace of the open junction and continue moving into the deeper reaches of the interspace by circumventing the closed fusions. These macular fusions thus subdivide rather than seal the ependymal interspace.

Part A Project Description (cont'd)

Proposed Course of Project: This paper has been accepted for publication in the Journal of Cell Biology.

Part B included: No

Serial No. NDB(I)-62 LNS/NC 927
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Fine Structure of Viral-induced Ependymomas

Principal Investigator: Milton W. Brightman

Other Investigators: R. Kirschstein and P. Gerber

Cooperating Units: Division of Biologic Standards
Laboratory of Viral Immunology

Man Years (computed for the 12 month period)
Total: 0.2
Professional: 0.2
Other: 0.0

Project Description:

Objectives: To compare the structure of normal and neoplastic ependymal cells.

Methods Employed: New-born Syrian hamsters are inoculated intracerebrally with SV_{40} virus. Approximately 100 days later, ependymomas have developed and the central nervous system of each animal is fixed in situ by vascular perfusion with buffered osmium tetroxide. Sections are examined with the electron microscope.

Major Findings: Where tumor cells are organized to form rosettes, their inter-relationships are similar to the normal ependyma where apical terminal bars are comprised, most commonly, of a luminal zonula adhaerens followed immediately by a zonula (or macula) occludens. Similarly localized fusions or maculae occludentes occur between adjacent tumor cells arranged in a rosette. These fusions occur at discrete sites and so compartmentalize the irregular intercellular spaces of the tumor.

Significance: Where tumor cells occur in loose array, their cytoplasm is highly evaginated; few or no specialized junctions link these cells of the presumably invasive portion

Part A Project Description (cont'd)

Proposed Course: This project has been temporarily discontinued. The wait of 100 days before learning whether or not the viral inoculation has been successful in producing a tumor makes it necessary, for the time being, to stop further work. It is intended to continue the study within the year.

Part B included: No

Serial No. NDB(I)-63 LNS/NC 1051
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The distribution within the brain of ferritin injected intraventricularly

Principal Investigator: Milton W. Brightman

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: To examine the distribution within the brain parenchyma of ferritin injected into the cerebrospinal fluid.

Methods Employed: A suspension of dialyzed, sterile ferritin in varying amounts is injected into the lateral or IV ventricle. After periods of 10 to 180 minutes the ependyma is fixed by either vascular perfusion or by infusion directly into the ventricles of osmium solutions.

Major Findings: The "submicroscopic" pericellular spaces of the brain are diffusion channels and not merely potential spaces. Micelles cross the glial border of the cerebellar subarachnoid space and parenchymal capillaries by moving across glial cytoplasm. Once inside the parenchymal interspace, however, molecules are free to reach the plasmalemma of glial and neuronal cells without further crossing glial processes.

Significance: The pericellular spaces, about 200Å wide, within the brain parenchyma are true diffusion channels along which substances can move.

Part A Project Description (cont'd)

Proposed Course of Project: The manuscript is to be submitted for publication in the Journal of Cell Biology.

Part B included: No

Serial No. NDB(I)-63 LNS/NC 1052
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Electron microscopic study of neurosecretion in the mammalian hypothalamus

Principal Investigator: M. Mazzuca

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 0
Professional: 0
Other: 0

This project is terminated. Dr. Mazzuca left the NIH and returned to France.

Serial No. NDB(I)-63 LNS/NC 1053
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Fine structure of the surface of the olfactory mucosa

Principal Investigator: Thomas Reese

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 0.2
Professional: 0.2
Other: 0.0

Project Description:

Objectives: To study the structure of the surface of the olfactory mucosa.

Methods Employed: Living olfactory mucosa from the frog was examined with phase and reflected light microscopy. For electron microscopy the mucosa was preserved so as to maintain intact the structures which were seen with the light microscope. Some of these structures were examined in serial sections.

Major Findings: Cilia originating from dendrites of bipolar olfactory neurones are up to 200 microns long, contain only single fibers in the major portion of their shafts, have vesicles between the ciliary membrane and the fibers in the shafts, and have divisions in their shafts bridged by a double fold of ciliary membrane. A complex system of centrioles and basal bodies is found in the tips of the dendrites. Some centrioles appear to be associated with basal bodies while others lie free in the cytoplasm. The supporting cells have a villous border and contain granules which appear to be secreted into the mucus covering the epithelium.

Part A Project Description (cont'd)

Significance: The presence of numerous specialized ciliary tips near the surface of the mucus covering frog olfactory epithelium suggests that these cilia may have a role in the responses of the epithelium to odors. The complicated system of centrioles at the origins of the cilia suggests a constant involution and evolution of these cilia. The supporting cells have a role in determining the composition of the mucus which bathes these cilia.

Proposed Course: This project has been completed. A complete report on frog olfactory epithelium will appear in the May issue of the Journal of Cell Biology. A comparative study of human olfactory receptors has been initiated.

Part B included: No

Serial No. NDB(I)-65 LNS/NC 1229
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Fine structure of the ventral cochlear nucleus

Principal Investigator: Thomas Reese

Other Investigators: G. L. Rasmussen

Cooperating Units: Section on Functional Neuroanatomy

Man Years (computed for the 12 month period)
Total: 0.3
Professional: 0.3
Other: 0.0

Project Description:

Objectives: To study the fine structural alterations in the synapses and nerve cells of the cochlear nucleus at different post-operative periods following destruction of the spiral ganglion.

Methods Employed: One cochlea in each chinchilla is carefully removed and the animal sacrificed at different post-operative time intervals. The brain is then perfused with buffered osmium tetroxide to fix it for electron microscopy.

Major Findings: Insufficient experimental material has been studied to report major findings at this time.

Significance: This study should provide much needed information concerning the ultrastructural alterations that accompany deafferentiation of neurons.

Proposed Course of Project: Dr. Reese will be leaving the NIH in September 1965 and will continue this project at Harvard Medical School in collaboration with Dr. Rasmussen.

Part B included: No

Serial No. NDB(I)-65 LNS/NC 1230
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Morphology of monoamine-containing neurons

Principal Investigator: Nicholas J. Lenn

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)

Total:	1.0
Professional:	1.0
Other	0.0

Project Description:

Objectives: Nerve cells of various parts of the brain were studied in order to discover the morphological aspects of the localization of monoamine within these cells. There were no special ultra-structural characteristics in normal and drug-treated animals and the possibility of auto-radiographic localisation is being explored.

Methods Employed: Rat brain tissue has been fixed with solutions of osmium tetroxide or glutaraldehyde, and embedded in epoxy resin. Thicker sections are studied with the light microscope after alkaline toluidine blue staining. Thin sections from selected regions were examined with the electron microscope after staining with heavy metal salts. For autoradiography, various methods of tissue handling, and several photographic emulsions are being used to study localization of tracer amounts of radioactivity, introduced in vivo in the form of metabolic precursors of monoamines.

Major Findings: The normal fine structure of the locus coeruleus, nucleus dorsalis raphes, area ventralis tegmenti, and substantia nigra, with particular attention to the neuronal perikarya of these regions, has been elucidated. Statistical estimates of the number of

Part A Project Description (cont'd)

granular vesicles in the nucleus dorsalis raphes before and after pargyline administration have been made on the basis of random samples. The autoradiographic studies are still in a preliminary stage.

Significance: The association of monoamine content with electron microscopically demonstrable structures is well established in the peripheral nervous system and the adrenal medulla. The usefulness of autoradiography in confirming this association was established in previous studies from this laboratory. The possibility that monoamines in neuronal perikarya of the central nervous system are associated with other cytoplasmic structures is important in questioning any generalization about the storage forms of monoamines. The present studies may also illuminate other problems concerning these hypothetical neurotransmitters, such as their locus of synthesis.

Proposed Course of Project: The autoradiographic studies will be continued. Various radioactive compounds will be surveyed by light microscopic autoradiography, and appropriate ones will be restudied with the higher resolution of electron microscopic autoradiography. A manuscript has been completed concerning the ultrastructure of the monoamine containing neurones.

Part B included: No

Serial No. NDB (I)-65 LNS/NC 1231
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Fine structure of the olfactory bulb

Principal Investigator: Thomas Reese

Other Investigators: M. W. Brightman

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 1.0
Professional: 1.0
Other: 0.0

Project Description:

Objectives: To study the structure of the olfactory bulb in the rat with the electron microscope.

Methods Employed: Olfactory bulbs were prepared by perfusing the brain with buffered osmium. Thin sections from these brains were examined with the light and electron microscopes.

Major Findings: The major pathways in the superficial layers of the olfactory bulb were identified at the fine structural level. Synapses in the various layers were studied in detail and a novel system of synapses between dendrites was found. Also, the small periglomerular neurones were found to give use to sheet-like processes which engulfed other periglomerular neurones.

Significance: An understanding of the anatomy of the olfactory bulbs in the finer detail possible with the electron microscope forms an important background for the many recent neurophysiological studies on the olfactory bulb. The identification of a system of dendo-dendritic synapses here helps to explain and lends support to some recent work in which inhibitory dendo-dendritic interaction is reported.

Part A Project Description (cont'd)

Proposed Course of Project: Preparation of a manuscript for publication of the data listed above. Examination of the deeper layers of the olfactory bulb is in progress.

Part B included: No

Serial No. NDB(I)-6 5 LNS/NC 1232
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The structural effects of the monoamine oxidase inhibitor Pargyline on the nerve endings of rat vas deferens

Principal Investigator: Keith C. Richardson

Other Investigators: I. A. Michaelson

Cooperating Units: Laboratory of Chemical Pharmacology, National Heart Institute

Man Years (computed for the 12 month period)
Total: 0.5
Professional: 0.5
Other: 0.0

Project Description:

Objectives: To investigate the effect of Pargyline on the granular and nongranular vesicles of the nerve endings in rat vas deferens in combination with assay of the norepinephrine content of the vas deferens after various dosages at suitable time intervals.

Methods Employed: Standard electronmicroscopical techniques of osmium tetroxide fixation and epoxy resin embedding, quantitative measurement of the numbers of granular and nongranular vesicles per unit area of nerve ending cytoplasm (minus area of mitochondria) in 800 separate electronmicrographs taken at random from 17 control and Pargyline injected rats. Chemical assay (Michaelson) of norepinephrine in ug/g of whole vas deferens from the contralateral side of each animal.

Major Findings: According to de Robertis and Iraldi, monoamine oxidase inhibitors cause a profound increase in the numbers of granular vesicles in the sympathetic nerves of the rat pineal. This could not be confirmed for the vas deferens. Whereas no assays were recorded for the pineal, Pargyline caused a rise in vas deferens

Part A Project Description (cont'd)

norepinephrine of about 25 per cent. This was accompanied by an increase in the total number of vesicles per unit areas of axon, but the effect was not significant for the numbers of granular vesicles.

Significance: The results make some contribution to the problem as to whether nongranular vesicles, as well as the dense core granular vesicles, contain norepinephrine, but it is clear that attempts to intensify the 'labelling' of adrenergic nerves by increasing their granular vesicle content cannot be achieved by monoamine oxidase inhibition. This is also the case for the adrenergic neurones of the C.N.S. (N. Lenn, this laboratory), despite a large increase in catecholamine fluorescence recorded by Swedish investigators.

Proposed Course of Project: A manuscript of these findings is in course of preparation.

Part B included: No

Serial No. NDB(I)-65 LNS/NC 1236
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

PART A

Project Title: Fine structure of the frog parathyroid gland

Principal Investigator: D. C. Rogers

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 0.5
Professional: 0.5
Other: 0.0

Project Description:

Objectives: To study the ultrastructure of the secretory cells of the frog parathyroid gland and the mode of release of the secretion into the blood stream.

Methods Employed: Fixation by perfusion through the heart with glutaraldehyde followed by osmium tetroxide; embedding in araldite epoxy resin; examination of ultra thin sections under the electronmicroscope.

Major Findings: The glandular tissue contained two cell-types, the 'dark' and 'light' cells. The main parenchyma consisted of 'dark' cells and the sub-capsular vascular plexus was surrounded by 'light' cells. Evidence was found for associating 'dark' cells with the active secretory phase and 'light' cells with the inactive phase. Secretory granules in these cells could not be traced outside the cells within intercellular channels communicating with the sub-endothelial spaces. The view that secretory granules are discharged in toto across the endothelial cell cytoplasm could not be confirmed.

Significance: This work is a contribution to the problems surrounding the mode of formation and secretion of parathormone which at present remain unsolved.

Part A Project Description (cont'd)

Proposed Course of Project: The work has been concluded and the investigator has returned to the Department of Zoology, University of Melbourne, Australia. A manuscript entitled "An electron microscope study of the parathyroid gland of the frog (rana clamitans)" is to be submitted for publication in Ultrastructural Research.

Part B included: No

Serial No. NDB(I)-62LNS/EE 931
1. Neuroanatomical Sciences
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The effect of neuropharmacological agents on the developing chick embryo.

Principal Investigator: Alfred J. Coulombre

Other Investigator: Stanislaw Jajszczak

Cooperating Units: None

Man Years (computed for the 12 mo. period)

Total:	0.6
Professional:	0.6
Other:	0.0

Project Description:

Objectives: We seek to assess short term and long term alterations in morphology, physiology, and behavior when drugs and other substances are administered during embryonic life.

Methods Employed: The use of a syringe driver and a polyethylene microcatheter developed in this laboratory permits continued, programmed or acute injection of solutions into the vascular compartment of the chick embryo.

Major Findings: Due to an early lead in this project attention was focused upon the effects of sugars (glucose, galactose, zylose) upon the developing lens of the chick embryo. Investigation yielded the following results:

1. All of these sugars were capable, when maintained at high concentrations in the blood by continuous infusion into the vascular compartment of the chick embryo, of producing cataractous changes. In all instances the nuclear portions of the lens were spared, and maximal damage was seen in the young fibers in the lens cortex and in the lens suture regions.

2. When sugar infusion was stopped to allow blood sugar levels to return to normal, the disintegrating lens fibers slowly disappeared from the lens which subsequently achieved complete transparency and was normal

in every way except that an unusual arrangement of lens fibers revealed by histology attested to the former presence of the lesions in the lens.

3. Only small d-sugars were capable of bringing about these changes, and small l-sugars proved ineffective in this respect. It thus appears that the effect is a specific one.

4. These results indicate clearly the possibility that prolonged elevation of blood sugar during embryonic life is one possible source of congenital cataract.

Significance: These findings give strong experimental support to the contention that elevated maternal blood sugar may be a causal factor in the development of cataracts in embryos and fetuses. It also points the way for future studies in which agents may be sought to counteract this (and possibly other deleterious effects) of elevated blood sugar during embryonic life.

Proposed Course of the Project: Revised objectives of this project have been achieved and the results are being prepared for publication. The project has been terminated.

Part B included: No.

Serial No. NDB(I)-62LNS/EE 932
1. Neuroanatomical Sciences
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The development of the pigmented epithelium of the retina.

Principal Investigator: Alfred J. Coulombre

Other Investigator: Jane L. Coulombre

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 0.6
Professional: 0.6
Other: 0.0

Project Description:

Objectives: Our present goals are: (1) to determine quantitatively the distribution in time and space of cell division, cell death, and cell enlargement, in the pigmented epithelium of the developing chick embryo; (2) to identify factors which affect these three variables and to evaluate the role of these factors in adjusting the dimensions of the pigmented epithelium to the geometry of the developing eye; (3) to determine which areas of the pigmented epithelium are capable of regenerating neural retina and lens; (4) to determine the time during development at which these capacities are lost; and (5) to identify these changes which occur in the pigmented epithelium at the time in development when it loses regenerative capacity.

Methods Employed: A battery of techniques were listed in project report No. NINDB-NS-EE-16 (1962). In addition to these we have added a surgical procedure especially developed in this laboratory. At four days of incubation the vitreous body and neural retina are removed from the eye of the chick embryo through a limbic incision. This removes the pigmented epithelium from the influence of the neural retina. Other tissues can be inserted into such an eye to test their ability to guide the pigmented epithelium in its regeneration of neural retina or other structures.

Major Findings: As a result of pilot experiments using chick embryonic material the objectives of this project were slightly altered in order to take advantage of promising leads. The studies which resulted led to the following conclusions:

1. The growth of the pigmented epithelium is contributed to by mitosis only very early in development. By the fourth day of incubation the pigmented epithelium of the chick embryo has most of the cells which will constitute it throughout life.

2. The increase in area of the pigmented epithelium in such a way that it corresponds in area at all stages in development with the adjoining neural retina is achieved by the enlargement of the individual cells. This cellular hypertrophy is most marked at the posterior pole of the eye. As a consequence, there is, at most stages in development, a gradient in cell size in this tissue from large, at the posterior pole, to small, at the cup margin. The distribution of sidedness in the cells of this simple, low cuboidal epithelium corresponds to topological expectations.

3. The expansion of the pigmented epithelium is driven by the accumulation of vitreous substance within the vitreous cavity of the eye, and it is in this way that its area is made to match that of the neural retina at each stage in development.

4. The pigmented epithelium of the chick embryo is capable of regenerating neural retina both as sheets from its margin, and as patches deep within the optic cup. Such regeneration can be initiated simply by separating the neural retina physically from the pigmented epithelium. However, regeneration of neural retina from pigmented epithelium will not proceed in the complete absence of neural retina. This inductive action of neural retina upon the pigmented epithelium can be substituted for by embryonic otocyst, but not by another neural tissue, the optic tectum.

Significance: Some vertebrates (the Salamandridae) can regenerate a neural retina throughout life. In such cases the regenerated retina makes functional connections. It appears from these studies that the embryos of other vertebrates may possess this capacity during early development and lose it at a particular stage. We know when this capacity is lost in the chick embryo. The study of changes occurring during that period in development may bring us closer to an ability to control the regeneration of tissues such as the neural retina. Progress has already been made in this direction.

Proposed Course of the Project: The results of these investigations are being incorporated into two papers, one of which has already been

submitted for publication, and accepted. Since the objectives of the project have been achieved, it has been terminated.

Part B included: Yes.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Coulombre, J. L. and Coulombre, A. J.: Regeneration of Neural Retina from the Pigmented Epithelium in the Chick Embryo. Develop. Biol. (in press).

Honors and Awards relating to this project:

None

Serial No. NDB(I)-62LNS/EE 933
1. Neuroanatomical Sciences
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The growth and shaping of the lens of the eye.

Principal Investigator: Alfred J. Coulombre

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 mo. period)
Total: 0.6
Professional: 0.1
Other: 0.5

Project Description:

Objectives: The role played by the lens in the eye in accomodation depends upon its refractive index, its volume and its shape. The growth (change in volume) and the change in shape of many parts of the eye (pigmented epithelium, choroid, sclera, and cornea) depend indirectly upon mechanical forces generated by a growing vitreous body. It was the purpose of this investigation to determine to what extent the developmental increase in the volume of the lens is similarly dependent, and to what extent mechanical tensions transmitted through the zonular fibers determine the final shaping of the lens.

Methods Employed: Drainage tubes inserted through the eye walls of early embryonic eyes will be used to modify the growth rate of the eyes as well as to relieve tension on the zonular fibers during that period of development during which the lens is being shaped. A capillary method for determining minute volumes was used to measure the volumes of a normal developmental series of the chick embryo lenses, as well as to measure the volumes of lenses from eyes which have been made to grow at a reduced rate. A filar micrometer fitted to a dissecting microscope was used to measure the major axial dimensions of appropriately oriented lenses from both untreated and intubated eyes at different embryonic ages. Planimetric methods were used to record the changes in area of suitably oriented axial sections of embryonic lenses from both the untreated and experimental categories.

Major Findings: By introducing minute drainage tubes through the eye wall of four-day chick embryos it has been possible to drain the vitreous substance from the eye as it forms, to prevent in this fashion the increase in intraocular pressure, and, as a result, to prevent expansion of the outer coats of the eye wall. As a consequence, eyes treated in this manner remain microphthalmic and can be made to grow at widely differing rates. By using this technique it is possible to uncouple the growth of the outer coats of the eye wall from that of the lens. Quantitative measurements revealed that the lens grows at a normal rate despite the fact that other elements in the eye (with the exception of the neural retina) have been made to grow at subnormal rates. The growth of the lens is, therefore, not tied in any obligatory way to the rate of growth of the outer coats of the eye wall (excepting the neural retina).

Significance: The eye is a precision optical instrument which, when it has completed its morphogenesis, must be appropriately shaped in all of its parts so that a focused optical image will be formed at the level of the rods and cones. It is becoming increasingly evident that some structures in the eye are dependent upon the growth of the vitreous body for their own growth, while others (e.g., the neural retina) are relatively independent of the growing vitreous body. In order to assess the mechanisms which are responsible for the coordinated growth of the several parts of the eye it is necessary to know into what category each ocular structure fits. The present study is an attempt to determine to what extent two properties of the lens (its volume, and its shape) are determined by mechanical tensions generated by a burgeoning vitreous body. This study, and others in progress in this section, seek to establish in detail the sequence of events which is responsible for the establishment of a visual organ in which all of the parts are appropriately shaped and situated with respect to one another, so that the laws of optics are obeyed.

Proposed Course of the Project: The results of this investigation complete the objectives of the project and have been prepared as a scientific article which is ready for publication. The project has been terminated.

Part B Included: No.

Serial No. NINDB(I)-63LNS/EE 1058
1. Neuroanatomical Sciences
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Factors controlling the size, shape and position of the lens of the eye during embryonic development.

Principal Investigator: Alfred J. Coulombre

Other Investigators: Jane L. Coulombre

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 0.9
Professional: 0.4
Other: 0.5

Project Description:

Objectives: In order that the eye may function properly as an optical instrument it is essential that the size, shape and orientation of the lens be precisely regulated with respect to other ocular tissues. We seek to identify the mechanisms which establish these relationships early in embryonic development, and which keep them continually adjusted as development proceeds.

Methods Employed: Microsurgical procedures have been developed in this laboratory which permit the removal of the lens at any stage during embryonic development. In addition the lens can be reinserted into the eye in any orientation which is desired. The operation also allows substituting two or more lenses in the place of a single lens. Finally, the lenses of other species of animals can be substituted for the lens of the chick embryo, and the consequences of this substitution followed during later development.

Major Findings:

1. There is a powerful mechanism which operates to control the size, shape, relative position, and orientation of the lens of the eye as it develops. This mechanism was demonstrated by deliberately disorienting lenses in early embryonic eyes and following the subsequent internal reorganization of these lenses. Single lenses which have been removed

from the eye and returned to it in unusual orientations reorganize in such a fashion that they become appropriately organized in cellular architecture, in size and in shape, in a direction appropriate for the eye of the host.

2. Lens epithelium attached to its adjoining lens capsule is capable, when placed in the pupillary opening of eyes from which the lens has been removed, of reconstituting an entire lens which is appropriately oriented.

3. When a single lens of a chick embryo at five days of incubation is replaced by two five-day lenses in any one of several orientations, the two lenses change their internal reorganization, their size, and their shape in such a manner that their combined mass has the appropriate size, shape and orientation appropriate to the eye of the host during subsequent stages of development.

4. The mechanism which operates to orient the lens of the chick embryo is not class specific since epithelia from mouse embryonic lenses will grow, elaborate lens fibers, and differentiate into lenses which are incapsulated, and appropriately oriented with respect to the host eye.

5. One of the factors responsible for the control of lens fiber differentiation, lens size, and lens orientation, is attributable to the neural retina. From studies in another laboratory it is clear that this factor, or complex of factors, is water-soluble and diffusable.

Significance: These studies not only increase our understanding of the mechanisms which control the orderly development of eye parts in such a way that the eye develops a geometry compatible with its optical function, but they also open the way for possible elective control of lens development and lens regeneration.

Proposed Course of the Project: The results of this investigation are being incorporated in a minimum of three papers. One of these papers has already been published, and two more are in preparation. These reports terminate this project. The original objectives of this project have all been achieved. Valuable additional information has also resulted from this undertaking, in addition to useful leads which will serve as the basis of future projects.

Part B Included: Yes.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

Coulombre, A. J. and Coulombre, J. L.: Lens development. I. Role of the lens in eye growth. J. Exp. Zool. 156: 39-48, June 1964.

Honors and Awards relating to this project:

None.

Serial No. NDB(I)-63LNS/EE 1059
1. Neuroanatomical Sciences
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Neural fold closure.

Principal Investigator: Alfred J. Coulombre

Other Investigators: Stanislaw Jajszczak

Cooperating Units: None

Man Years (computed for the 12 mo. period)
Total: 0.6
Professional: 0.6
Other: 0.0

Project Description:

Objectives: We aim to determine what factors underlie the closure of the neural folds during early embryonic development. In these studies we seek to answer such questions as: For how long can the neural folds be prevented from uniting and yet retain their capacity to do so? Will the neural folds reunite if they are mechanically or chemically separated following closure? What are the consequences of preventing closure of the neural folds in different regions of the neuraxis or of reopening the folds once they have closed?

Methods Employed: The chick embryo is used. We employ three methods of manipulating closure of the neural folds: 1) The insertion of small pieces of gold foil at selected levels of the neuraxis just prior to closure of the neural folds in that region; 2) the application by micropipette of a range of enzymes (e.g. hyaluronidase, ribonuclease); 3) the use of special instruments that permit blunt dissection of the closed neural folds at different periods following their closure, and at different levels of the neuraxis. Animals treated in these ways are observed at successive stages following treatment.

Major Findings: By inserting small pieces of gold foil between the unclosed lips of the embryonic neural tube of the chick embryo, it was possible to prevent the closure of the tube at defined anterior-posterior levels. By studying the subsequent development of embryos treated in this fashion, and in addition, embryos in which the fused lips of the

neural folds were separated mechanically immediately following their closure, we have demonstrated the following:

1. The neural folds retain the ability to effect successful closure of the neural tube at all levels for only a brief period following the time in development at which they approach each other.

2. Failure of closure of the neural tube at any final level leads to overgrowth of the alar plate.

3. Prevention of closure of the neural folds frustrates the accumulation of cerebral spinal fluid in the ventricular spaces and spinal canal. Under these conditions the optic vesicle which evaginates from the diencephalic wall fails to expand completely, and either makes no contact with the surface ectoderm, or only tenuous contact. In cases where the expanding optic vesicle fails to contact the surface ectoderm no lens is formed, and anophthalmia results. In cases where contact is made, but where it is subnormal, microphthalmia results. Thus, in addition to mitotic factors and other mechanisms assuring the evagination of the optic vesicle, it is clear that intraventricular pressure plays a positive role in the successful expansion of the optic vesicle, and therefore in the subsequent normal development of the eye.

Significance: These studies have confirmed that interference with closure of the neural folds at any level of the neuraxis can precipitate spina bifida in the lumbo-sacral region with the highest incidence of closure defects observed in humans. In addition, a new factor has been discovered that contributes to the normal development of the vertebrate eye. It is clear that, at least in this form, intraventricular pressure within the early embryonic brain is important in fully expanding the embryonic optic vesicle and thus assuring normal development of the eye. Conversely, it is probable that some instances of microphthalmia may result from failure of closure of the neural tube, or even from delay in closure of the tube.

Proposed Course of the Project: The results of this investigation are in preparation in the form of a scientific article which will be submitted for publication. This will terminate this project with achievement of all of the major objectives and the uncovering of information not anticipated in the original design.

Part B Included: No.

Serial No. NDB(I)-63LNS/EE 1060
1. Neuroanatomical Sciences
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Corneal development

Principal Investigator: Alfred J. Coulombre

Other Investigator: Jane L. Coulombre

Cooperating Units: None

Man Years (computed for the 12 mo. period)
Total: 0.2
Professional: 0.2
Other: 0.0

Project Description:

Objectives: This is a very broad project aimed at gathering as much information as possible concerning morphogenesis, histogenesis, and cytogenesis of the developing vertebrate cornea. In the past this program has yielded information concerning those factors involved in the development of transparency in the cornea, in the determination of shape of the cornea, and in the physical basis of the interference pattern seen in the cornea when it is viewed between crossed polarizers.

Methods: This project utilizes an extremely broad and continually expanding array of techniques in an attempt to define in some detail the changes which go on in the developing vertebrate cornea. Among these techniques in the past have been: physical techniques for the measurement of light transmission through the embryonic cornea; microsurgical procedures for assessing the role of intraocular pressure in the development of the cornea, the role of the lens in the induction and maintenance of the embryonic cornea, etc.; nuclear tagging with tritiated thymidine in order to follow the population dynamics of the cells in the cornea; histologic, histochemical and cytologic methods; etc.

Major Findings: During the past year it was decided to abandon all but one of the objectives of this project. By removal of the embryonic lens of the chick embryo, and by its replacement in certain instances, it was possible to show that the inductive influence of the lens upon the cornea continues throughout an appreciable period of development, and is not confined to a short-lived inductive act early in development.

Significance: The finding which resulted from this project indicates clearly that the induction of the anterior epithelium of the cornea by the embryonic lens is not a transient influence, but one which involves the dependency of the developing cornea on the embryonic lens for a prolonged period during embryonic life. This would suggest that certain congenital defects of the cornea may be secondary to a primary involvement of the embryonic lens.

Proposed Course of the Project: With the successful achievement of this objective, which was published as part of a broader report, this project has been terminated.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

Coulombre, A. J. and Coulombre, J. L.: Corneal development. III. The role of the thyroid in dehydration and the development of transparency. Exp. Eye Res. 3: 105-114, June 1964.

Honors and Awards relating to this project:

None.

Serial No. NDB(I)-63LNS/EE 1061
1. Neuroanatomical Sciences
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: In vitro studies of lens differentiation.

Principal Investigator: Gordon W. Philpott

Other Investigators: Alfred J. Coulombre

Cooperating Units: None

Man Years (computed for the 12 mo. period)

Total:	1.1
Professional:	1.1
Other:	0.0

Project Description:

Objectives: a) To identify and study the developmental factors which influence the differentiation of the lens epithelial cells into lens fibers. b) To examine the mechanisms which control mitosis in the lens epithelium.

Methods Employed: Lens epithelia are removed from 4-18 day embryonic chick eyes, isolated and maintained either in the absence of, or the presence of, test tissues, in a variety of culture media utilizing several different tissue and organ techniques. Histological, histochemical, and cytological methods were applied to these tissues to determine the degree of lens fiber formation from epithelial cells. Such an approach permits the definition of those conditions which are necessary to support the differentiation of lens fibers from lens epithelial cells.

Also, lens epithelial cells cultured for 5 days can be returned to lenseless 5-day chick embryonic eyes. The effect of this reimplantation on these cells can be examined histologically.

In addition, the production of lens specific proteins by these cultured epithelial cells is being determined by immunochemical techniques including those of Ochterlony.

Major Findings: Explantation of embryonic lens epithelial cells into a completely defined culture medium (TC-199, N-16) is followed by simple maintenance of these cells without either their increase in thickness

or their entry upon a phase of cell division. If, however, such cultures are supplemented with small amounts of serum protein (several sources were used and the requirement did not seem to be specific) the lens epithelial cells quickly enter upon a phase of limited elongation during which they partially differentiate into lens fibers. Under these conditions the cells do not subsequently divide in the cultures. When protein supplementation was delayed for various periods of time it was determined that supplementation would no longer elicit elongation of the lens epithelial cells after they had been maintained for 48 hours in totally defined medium. Thus, the information necessary for the elongation of these cells had been exhausted in the interval. When protein supplements were made at, or following, 48 hours of culture in defined medium the lens epithelial cells entered upon a phase of active cell division. It is, thus, clear that lens epithelial cells:

a) have a limited amount of information which enables them to partially differentiate into lens fibers;

b) that this information can be exhausted under the conditions of the experiment within 48 hours;

c) that active cell division, like cell differentiation, requires protein supplementation, but that the ability to divide is not expressed until the cells have totally exhausted their capacity for partial differentiation into lens fibers. This transition occurs at about 48 hours in culture.

2. Lens epithelia which have been maintained in culture for varying periods of time up to 48 hours, with or without protein supplementation, maintain the ability to respond in a specific way to the eye environment by differentiating into a well-formed lens. This was demonstrated by returning such tissues to embryonic eyes from which the lenses had been removed. Under these circumstances the implants differentiate into well formed lenses with capsules, lens epithelia, and a posteriorly situated mass of lens fibers. Lens epithelia which were transplanted from tissue culture to the coelom of chick embryos showed no such differentiation.

These studies have provided an in vitro system which is capable of responding to the presence of a soluble factor arising from the neural retina which has been demonstrated in other studies. Thus, the way is opened for isolation and identification of the factor or factors arising from the neural retina which are essential for lens fiber elongation, for orientation of the lens during embryonic development, and for growth of the lens.

Significance: The data gathered so far suggests that there are several (and perhaps many) factors regulating the differentiation of lens epithelial cells into lens fibers. The importance of the neural retina

is emphasized but the chemical factor or factors responsible may not arise solely from the neural retina. Inhibitory factors such as mechanical tension may play an important role in regulating lens epithelial cell differentiation. These studies, together with others current within this Section, are increasing our knowledge of the mechanisms which control the size, shape, position, and orientation of the lens in the vertebrate eye.

This work has provided us with an *in vitro* test system capable of sensing the presence of retinal-lens factor. This offers hope of concentrating, isolating, characterizing, and identifying this factor. Should these steps (to be attempted in another project) prove possible one of two remaining obstacles to the controlled regeneration of functional lenses in vertebrate eyes would be removed.

Proposed Course of the Project: The results of this study have been incorporated into two papers, one of which has already been accepted for publication. This project has been completed, having achieved all of its objectives, and in addition, yielding some results that were not anticipated in the original plans.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

Philpott, G. W. and Coulombre, A. J.: Lens development. II. The differentiation of embryonic chick lens epithelial cells *in vitro* and *in vivo*. *Exp. Cell Research*, Stockholm (in press).

Honors and Awards relating to this project:

None.

Serial No. NDB(I)-63LNS/EE 1062
1. Neuroanatomical Sciences
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Histogenetic studies of the central visual centers in chicks.

Principal Investigator: G. Robert DeLong

Other Investigators: Alfred J. Coulombre

Cooperating Units: None

Man Years (computed for the 12 mo. period)
Total: 1.1
Professional: 1.1
Other: 0.0

Project Description:

Objectives: We seek to describe the embryological development of the cytoarchitecture of optic tectum of the chick embryo as a favorable instance of the general development of the brain. The embryonic cytoarchitecture will, so far as possible, be correlated with the mature cytoarchitecture and with the function of this region.

The further objective is to elucidate the manner in which specific synaptic relationships are established between different portions of the developing nervous system. In particular, we seek to study, in the retina-tectum system, how these synaptic specifications are established during embryological development.

Methods Employed: A battery of techniques is being used in this study. These include: autoradiography, neurological stains (reduced silver and Golgi preparations), and experimental embryological methods involving ablations, grafting, and the use of chemical agents on chick embryos.

Principal Findings:
1. By use of silver stains and other histologic techniques, the cytoarchitecture of the developing optic tectum of the chick embryo has been followed during pre-hatching development. This phase of the study has revealed:

a) The sequence in which the several cellular and fiber layers of the tectum make their appearance;

b) A description of the maturation of the tectum by area during the course of development;

c) The temporal and spatial aspects of invasion of the tectum by ganglion cell axons from the neural retina.

2. By introducing pulses of tritiated thymidine some insight has been gained into the time and place of origin of the definitive neuroblasts that constitute the cellular layers of the optic tectum.

3. By introducing lesions at known points in the neural retina of the early embryonic chick it was possible to map the distribution of the fibers which arise from this structure on the surface of the optic tectum. These studies revealed, in addition, that the topographic map on the surface of the optic tectum arises, at least in this species, independently of the similar topographic map on the surface of the neural retina.

Significance: These studies have revealed for the first time that the tectal topographic map representing the neural retina develops in complete independence of the latter and is not under the influence of the ingrowing ganglionic cell axons of the retina. Thus, the study clearly indicates that, at least in this instance, the patterns of organization typical of different portions of the same neuronal system can develop autonomously and in relative independence of other portions of the system. It appears that appropriate neuronal interconnections between cognate systems such as the neural retina and the optic tectum occur only secondarily. This draws attention to the basic problem of the forces which guide interconnecting axons in such a manner that they find a way to appropriate destinations in the target tissue. Thus the results achieved in this project carry us a step closer to understanding the manner in which the genetic information which encodes the structure of the nervous system becomes actualized during embryonic development.

Proposed Course of the Project: Thus this project has been successfully completed, and has achieved all of its objectives. The results have been prepared in the form of a scientific article which will soon be submitted for publication. The project will be terminated on 1 July 1965.

Part B Included: No.

Serial No. NDB(I)-65LNS/EE 1233
1. Neuroanatomical Sciences
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Morphogenesis of the inner ear.

Principal Investigator: Robert J. Ruben

Other Investigator: Alfred J. Coulombre

Cooperating Units: None

Man Years (computed for the 12 months period)
Total: 1.0
Professional: 1.0
Other: 0.0

Project Description:

Objectives: This project has two goals: a) To establish the step-by-step sequence by which the several portions of the inner ear are formed, and to trace in detail the histogenesis of both the cochlear and vestibular portions of the inner ear; b) To identify the time of onset and the nature of genetic defects which develop in the inner ears of a series of mutant forms in the mouse.

Methods Employed: Inbred strains of mice will be used. Routine histological and histochemical procedures will be applied. In addition, tritiated thymidine will be pulsed into pregnant female mice at different stages during gestation. By serially sacrificing the embryos and fetuses of such animals, and by processing the tissues of their inner ears autoradiographically, we plan to reconstruct, a step at a time, the histogenetic history of the cochlear and vestibular organs. As occasion arises, microsurgical methods will be employed in chick and mouse embryos in order to elucidate the dynamics of morphogenesis of inner ear.

Major findings: This project has only recently been activated, but it has already been possible to show the adequacy of the techniques for our stated objectives.

Significance: Inbred strains of mice exist in each of which single point mutations have been isolated which affect one or another aspect of the development of the inner ear, or the central auditory pathways. It is notable that these readily available mutant forms mimic, in both their

gross behavior and in the clinical picture which develops, similar defects known to occur in man. The utilization of this material holds great promise not only in unraveling the step-by-step interactions among the tissues of the ear which are responsible for its orderly morphogenesis and histogenesis, but also afford the very best opportunity for understanding the nature of auditory and vestibular defects of both genetic and teratogenic origin, and of pinpointing the specific steps in development at which the defects originate.

Proposed course of the Project: This undertaking has been divided into two phases: a) We will first establish a base line of normal histogenesis and morphogenesis for the inner ear of the mouse; b) Utilizing the information from the initial phase of the study, we will investigate the manner in which ear defects develop in a carefully selected range of mutant forms in which the inner ear is affected.

If time allows, experimental embryological techniques will be used to further elucidate the way in which the several components of the inner ear interact during development to assure an orderly morphogenesis. It is anticipated that this project will have been completed by 1 July 1966.

Part B included: No.

Serial No. NDB(I)-65LNS/EE 1234
1. Neuroanatomical Sciences
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Ocular Morphogenesis.

Principal Investigator: Alfred J. Coulombre

Other Investigator: Jane L. Coulombre

Cooperating Units: None

Man Years (computed for the 12 mo. period)
Total: 0.2
Professional: 0.2
Other: 0.0

Project Description:

Objectives: As a result of work in the Section on Experimental Embryology, a wealth of information has accumulated concerning the mechanisms which control the step by step development of the tissues of the vertebrate eye in such a way that they have sizes, shapes, positions, orientations, and differentiated properties appropriate to the optical function of the eye. This orderly development is brought about by a precisely timed series of interactions among the tissues of the developing eye. It is our purpose to continue to determine when such influences are mediated, which tissues give rise to them, which tissues are capable of responding to specific influences, and the nature of the responses in each instance. Further, the time has come when it may be possible to partially isolate and characterize the chemical substances responsible for these interactions.

Methods Employed: Microsurgery of chick embryos will be used to remove, increase the amount of, or experimentally rearrange the relationships between the several tissues of the developing eye. Tissue and organ culture will be employed in assessing the interactions between pairs of ocular tissues derived either from different parts of the eye, from different stages in development, or both. Routine histological and histochemical procedures will be employed to determine the results of these procedures.

Major Findings: The findings which form the background for this study were the results of previous projects undertaken in the Section on Experimental Embryology. While a large number of interactions among ocular tissues have already been identified, attention will be here focused on only two specific interactions.

1. The retina-lens factor: We have been able to demonstrate that a water-soluble, diffusible factor arises from the embryonic neural retina and is responsible for maintenance of the growth of the lens, for continually adjusting the orientation of the lens with respect to the rest of the eye, and for permitting differentiation of lens cells into lens fibers.

2. The retina-pigmented epithelial factor: Similarly, an influence originating in the neural retina has been shown to be responsible for initiating regeneration of neural retina from the pigmented epithelium in the early embryonic chick eye.

Significance: These two examples are representative of individual steps in the orderly cascade of influences among the tissues of the developing eye which account for the observation that the morphogenesis of this organ is held within the relatively narrow geometrical tolerances required by the laws of optics. In each of the two instances mentioned, *in vitro* and *in vivo* systems have been developed that are capable of detecting the presence of the substances involved. Thus, while the technical difficulties may prove to be considerable, the way is open in principal for the isolation, characterization, and eventual identification of the active substances. While "morphogens" have been suspected in many developing systems, none has yet been isolated. Access to these substances would greatly increase our ability to manipulate not only embryonic tissues but also tissues in the adult organism. For example, the ability to isolate and utilize the retina-lens factor would remove one of the two remaining obstacles to our ability to regenerate functional lenses in adult vertebrate eyes.

Proposed Course of the Project:

1. Preliminary experiments have indicated that the retina-lens factor is not species specific. We will seek to further characterize the species specificity of this factor.

2. The potency of neural retinae of different embryonic ages will be tested with respect to the ability to support lens differentiation and to maintain lens orientation.

3. Neural tissues other than neural retina will be tested for their ability to support differentiation and to maintain lens orientation.

4. Using an *in vitro* system with demonstrated sensitivity to the retinal-lens factor, which was developed in this laboratory during the preceding year, we will attempt to concentrate and characterize this factor.

5. A similar series of investigations will be initiated for the retinal-pigmented-epithelial factor.

Part B included: No.

Serial No. NDB(I)-65LNS/EE 1235
1. Neuroanatomical Sciences
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Histogenesis of the neural portions of the visual system as related to the onset of function.

Principal Investigator: Alfred J. Coulombre

Other Investigators: Paul N. Anderson and Edward R. Walpow

Cooperating Units: None

Man Years (computed for the 12 mo. period)
Total: 0.1
Professional: 0.1
Other: 0.0

Project Description:

Objectives:

1. To determine the sequence in which the neurons of the neural retina and the optic tectum become electrically active as embryonic development proceeds.

2. To determine the mitotic history, site of origin, and pathways of migration of the several neuronal components of the retina and optic tectum during the course of their development.

3. To determine by experimental intervention any effects which the several portions of the visual system exert one upon the other during embryonic development, with special attention to histogenetic influences, modifications in the pattern of development of electrical activity, and other types of trophic interactions.

Methods Employed:

1. Routine histological (including silver stains) and histochemical procedures will be employed to study the developing nervous system of the chick embryo.

2. Tritiated thymidine will be pulsed into chick embryos at different stages during development. After each pulse animals will be sacrificed seriatum in order to follow the histogenesis of the retina and optic tectum.

3. Surgical intervention during embryonic development will be used to remove specific portions of the visual system at known stages of development in order to assess the influence of one portion of the system upon another.

Major findings: Work recently completed in this section has already demonstrated that "topographic maps" develop separately and independently in the neural retina on the one hand and the optic tectum on the other. By means of appropriate connections made by the axons of the ganglion cells of the neural retina, these two topographic systems subsequently become appropriately interconnected as development proceeds. The current project, which will be activated 1 July 1965, will build upon these observations in further exploring the manner in which the visual system is constructed during embryogenesis.

Significance: By studies of this sort we are beginning to build a step by step flow sheet of the causal sequence responsible for the orderly development of the visual system. Such a flow sheet forms the basis for our understanding of how the genetic information governing the development of this portion of the nervous system becomes actualized during development. In addition, it provides a rational basis for understanding the abnormalities which develop when one or another step is interfered with either by genetic accident, or otherwise. In addition, a flow sheet of this sort makes clear the qualitatively or quantitatively different hazards that result from untoward influences on the developing system at different stages in its maturation.

Proposed Course of the Project:

1. An intensive histogenetic analysis using the methods outlined above will be undertaken commencing 1 July 1965.

2. Simultaneously electrophysiological techniques will be utilized to explore the electrical activity of the developing visual system.

It is anticipated that the objectives of this project will be achieved by 1 July 1967.

Part B Included: No.

Annual Report
July 1, 1964 through June 30, 1965
Laboratory of Neuropathology
National Institute of Neurological Diseases and Blindness

The Laboratory of Neuropathology, represented by the Section on Experimental Neuropathology, has terminated various investigations which provide new information about the organization of the central nervous system and about the changing reaction of cells along cerebral blood vessels during aging.

It was found that, in the normal brain, there is a fixed relationship between nerve cells and cells of the vascular walls, permitting an interaction. As the consequence of a neurogenic stimulus, mitotic division is induced in a cell along the vascular wall, and a new cell type, the microglia cell, is formed. This cell, because of its location, can be utilized as an alternate transport route between the blood vessel and the neuron. Since the rate of cell division was found to be reduced with increasing age, it was concluded that the ability to form alternate routes of transport is less in the older than in the younger organism.

A renewed investigation of strands of connective tissue between blood vessels revealed the presence of granular particles, indicating that these strands may serve as intervascular routes of transport. It was also disclosed that cells resembling mast cells are situated near these strands and also near oligodendrocytes. Since mast cells are known to contain many biologically active substances, such as serotonin, which affects the action of oligodendrocytes, these studies open new areas of research whereby the significance of various central nervous system elements under normal and pathologic conditions may be better understood. In order to determine the reaction of various cell types, with special emphasis on the problem of aging, a number of experiments have been performed, such as exercise of both untreated and corticosteroid treated animals, and slight and severe damage of cerebral tissue. Examination of this material is awaiting histologic preparation.

The aim of research projects in the immediate future will be to establish the conditions under which neuropathologic experiments must be carried out and to ascertain the patterns of normal organization, the criteria of functional and pathologic cellular changes, and the factors involved in maintaining normal central nervous function. Thus, some of the current controversial opinions in basic neuropathology may be clarified, and a better

understanding of the intricate mechanism concerned with normal functioning of the brain may further our knowledge about the pathogenesis of abnormal functioning of the brain due to temporary or permanent damage. Such projects will eventually entail utilization of the electron microscope and of histochemical techniques, which although pressingly needed, must await availability of other quarters, as planned with transfer to the new research building.

Serial No. NDB(I)-63 LN/EN 1063
1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The reaction of mesodermal cells in the central nervous system during senescence.

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)

Total:	0.5
Professional:	0.2
Other:	0.3

Project Description:

Objectives: To assess the effect of aging on the reaction of mesodermal cells of the central nervous system.

Methods employed: The development and incidence of mitotic cells were studied in tissues undergoing retrograde changes after transection of the facial nerve in rabbits varying in age from one month to three years and in six month old mice. Some rabbits were treated with cortisone.

Major findings: The following new observations were made: 1) The number of mitotic cells depends on the length of post-operative survival and the age of the experimental animal.

2) Mitotic cells in the vascular walls cause temporary narrowing of the capillary lumen.

3) When mitotic division in histiocytes is completed outside the vascular wall, microglia cells ensue.

4) Juxtavascular mitotic cells occur at specific sites where neuronal surface and capillaries meet.

Part A (cont.)

Significance: 1) The age of the experimental animal must be known in all studies concerned with the intensity of mesodermal reaction along cerebral blood vessels and of microglia cell proliferation in the brain.

2) Microcirculatory disturbances may interfere with reparatory phases of reactive tissue changes.

3) Histiocytes and microglia cells are distributed according to a specific pattern dictated by the spatial relationship between blood vessels and neurons.

4) According to microscopic evidence, a juxtavascular cell during mitotic division prepares the tissue so that maturation of daughter cells results in the formation of microglia cells.

5) Cortisone treatment reduces the incidence of karyokinesis, i.e. the action of cortisone is not restricted by the blood-brain-barrier.

Proposed course of project: Material obtained from cortisone treated animals subjected to transection of the facial nerve will be prepared by histologic techniques developed in this Section.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards,and Publications

Publications other than abstracts from this project:

Cammermeyer, J.: Juxtavascular karyokinesis and microglia cell proliferation during retrograde reaction in the mouse facial nucleus. Ergebn. Anat. Entwicklungsgesch. 38: 1-22, March 1965.

Cammermeyer, J.: Endothelial and intramural karyokinesis during retrograde reaction in the facial nucleus of rabbits of varying age. Ergebn. Anat. Entwicklungsgesch. 38: 23-45, March 1965.

Cammermeyer, J.: Histiocytes, juxtavascular mitotic cells and microglia cells during retrograde changes in the facial nucleus of rabbits of varying age. Ergebn. Anat. Entwicklungsgesch. 38: 195-229, March 1965.

Serial No. NDB(I)-62 LN/EN 942
1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Histochemical study of nerve cells undergoing retrograde changes.

Principal Investigator: Jan Cammermeyer

Other Investigators: Alden W. Dudley, Jr.

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 0.0
Professional: 0.0
Other: 0.0

Project Description:

Objectives: To discern the mode of glycogen deposition in neurons.

Methods employed: Rabbits are subjected to transection of the facial nerve, and the brain is fixed in situ and prepared according to methods developed in this Section.

Major findings: There are through the spinal cord and brain stem two types of motor nerve cells which have been found to react differently to cutting of the nerve fiber emanating from them. The two types of nerve cells are distinguished by varied histochemical composition.

Significance: The purpose of this study is to identify the biochemical basis for the difference in cell appearance and reaction and to determine the sequence of biochemical changes during acute changes of the nerve cells.

Proposed course of project: Microscopic examination of the histologic material which has been prepared by various histochemical enzyme digestion methods.

Part B included No

Serial No. NDB(I)-62 LN/EN 943
1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The effect of aging on the central nervous system.

Principal Investigator: Alden W. Dudley, Jr.

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 0.6
Professional: 0.6
Other: 0.0

Project Description:

Objectives: To establish what effect aging may have on cerebral neurons of the rabbit.

Methods employed: Materials prepared according to current methods of perfect fixation in situ are used to outline the boundaries of cytoplasm and nucleus of nerve cells. The size of various cell types, reproduced in drawings, is determined by an electronic scanner technique and the results subjected to statistical analysis by computer technique (contract with the National Biomedical Research Foundation, Silver Spring, Maryland).

Major findings: The results of a first series of 2000 neurons obtained by electronic scanning device are presently being analyzed. Another series of 3,600 cortical neurons is being subjected to electronic scanning analysis.

Significance: To obtain exact information about the effect of aging on size of neuronal cytoplasm and nuclei in two regions of the rabbit cerebrum.

Proposed course of project: Report of results and review of current concepts in manuscripts under preparation.

Part B included No

Serial No. NDB(I)-62 LN/EN 944
1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Hematologic control of primates.

Principal Investigator: Mrs. Margaret G. Johnson

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 0.6
Professional: 0.0
Other: 0.6

Project Description:

Objectives: To clarify the reason for poor health in primates.

Methods employed: Different primate species are regularly examined by various hematologic techniques; weight and dietary regimen are controlled.

Major findings: Many of the primates suffer from severe infections and aberrations of blood composition.

Significance: A careful hematologic examination is required prior to subjecting primates to experiments.

Proposed course of project: Regular check of primates and control of infectious condition.

A long term study of the hematologic status in healthy animals.

A long term study of the effect of varied dietary regimen and adjuvants on the hematologic status.

Part B included No

Serial No. NDB(I)-63 LN/EN 1064
1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Mitotic division of mast cells in the central nervous system.

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)

Total:	0.5
Professional:	0.2
Other:	0.3

Project Description:

Objectives: To determine whether mast cells of the central nervous system can be activated.

Methods employed: Retrograde changes of the facial nucleus are induced in rabbits by transection of the facial nerve. Some rabbits were treated with cortisone.

Major findings: 1) Mast cells undergoing mitotic division occur in the facial nucleus on the operated side.

2) Mast cells are depleted of their granular material after cortisone treatment.

Significance: There is an increased demand for biochemically active substances contained in mast cells during reactive changes of the brain, and their function is controlled by homeostatic factors such as cortisone.

Proposed course of project: Completed.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards,and Publications

Publications other than abstracts from this project:

Cammermeyer, J.: Reaction of mast cells identified in the rabbit brain. To be presented at the 41st Annual Meeting of the American Association of Neuropathologists, Atlantic City, June 12, 1965.

Serial No. NDB(I)-63 LN/EN 1065
1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Perfection of the perfusion technique for fixation in situ.

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 0.8
Professional: 0.3
Other: 0.5

Project Description:

Objectives: To eliminate fixation failures and to specify conditions of perfect fixation.

Methods employed: The rapidity of action of various fixatives has been tested.

Major findings: It is necessary to delay the autopsy after perfusion. With rapidly acting coagulant fixatives, the interval need not be so long as previously described (Exp. Neurol. 2: 379-405, 1960).

Significance: The time required for fixation can be shortened, whereby the entire procedure of histologic preparation can be speeded up. The risk of losing valuable material because of imperfect fixation is reduced. By a correct choice of solution, the occurrence of artifactual neuronal changes is minimized.

Proposed course of project: Final preparation of manuscript.

Part B included No

Serial No. NDB(I)-63 LN/EN 1066
1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Acute degenerative changes in the central nervous system.

Principal Investigator: Jan Cammermeyer

Other Investigators: Alden W. Dudley, Jr.

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 1.1
Professional: 0.4
Other: 0.7

Project Description:

Objectives: To define the sequence of changes in well-fixed materials.

Methods employed: Experimental microembolization is carried out in various animal species which at different post-operative stages are sacrificed by the perfusion procedure developed in this Section.

Major findings: Minute lesions are distributed in a manner which varies for each animal species.

Significance: In an attempt to appreciate correctly the significance of pathologic neuronal changes encountered in clinical neuropathologic material, it is necessary to study carefully tissues undergoing acute degenerative changes in experimental material prepared according to strict scientific standards.

Proposed course of project: Microscopic examination is awaiting the histologic preparation of already completed experiments.

Part B included No

Serial No. NDB(I)-63 LN/EN 1067
1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Intervascular strands of connective tissue fibers.

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 0.3
Professional: 0.1
Other: 0.2

Project Description:

Objectives: To assess extravascular routes of transportation in the central nervous system.

Methods employed: Histologic material is prepared by methods of fixation and staining as developed in this Section.

Major findings: Intervascular strands of connective tissue fibers in the brain are not homogeneous.

Significance: Microscopic evidence of pathways for transport of substances between adjacent blood vessels indicates that these intervascular strands can be helpful in providing dispersal of biochemically active substances.

Proposed course of project: Completed.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Cammermeyer, J.: Cerebral intervascular strands of connective tissue as routes of transportation. Anat. Rec. 151: 251-259, March 1965.

Serial No. NDB(I)-65 LN/EN 1237
1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Cytologic characteristics of microglia cells.

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years (computed for the 12 month period)

Total:	0.6
Professional:	0.2
Other:	0.4

Project Description:

Objectives: To define the cytologic characteristics of normal microglia cells in the rabbit cerebral cortex.

Methods employed: Histologic staining of perfused fixed animals of known age.

Major findings: Microglia cells occur throughout the central nervous system and assume different shapes depending on local cytoarchitectonic characteristics.

Significance: Clarification of controversial opinions about their occurrence and function.

Proposed course of project: To determine the appearance of microglia cells in animals of different species and of varying age and in experimental animals.

Part B included No

ANNUAL REPORT
July 1, 1964 through June 30, 1965
Laboratory of Neurophysiology, Intramural Research
National Institute of Neurological Diseases and Blindness

Wade H. Marshall, Ph.D., Chief

Increasing attention has been given in the Spinal Cord Section to the problem of synaptic transmission. This has involved three different aspects of the problem:

1) The basic mechanisms of synaptic transmission have been studied. A test of the most widely accepted model of chemical transmission at the synapse has been made by measuring cell impedance during the course of synaptic transmission. With a majority of monosynaptic excitatory synaptic potentials no measurable impedance change occurs although inhibitory synaptic potentials do produce such a change. These findings are consistent with an electrical mechanism of excitatory synaptic transmission and exclude the simplest model of chemical excitatory synaptic transmission. The findings do not rule out chemically mediated synapses located on the cell dendrites.

2) In view of this result and the increasing attention which is being given in a number of laboratories to the possible functional implications of the structure of dendrites, experiments were started which attempt to demonstrate the manner in which synaptic connections to a cell are distributed to the various dendritic branchings. The question is asked whether the manner in which various inputs to a cell are grouped or segregated affects significantly the way in which the cell integrates these inputs. The results available indicate that there is non-homogeneous dendritic distribution of synapses from different sources and that the distribution can alter the manner in which these synapses interact. Analysis of this data is as yet just begun.

3) The statistical properties of unitary synaptic events which occur in central neurons is being investigated. The behavior of the miniature synaptic potentials in the absence of stimulation indicates that they are not "random" events in the sense of being independent of one another. This information, coupled with the behavior of the miniature potentials under various conditions of stimulation, will be used to construct theoretical models for the regulation of cell firing.

Interaction between spinal motoneurons which appears to be electrical in nature has been demonstrated. The functional significance of this has not been shown as yet, however.

Evidence has been obtained that some degree of relationship exists between the electrical resistance of a motoneuron, its axonal conduction velocity and its synaptic excitability. This evidence correlates well with experiments of others showing the correlation between the conduction velocity of the axon of a cell and the way that cell participates in reflex activity.

Sensory coding in the auditory system is being examined in the cat by presenting a relatively wide variety of auditory stimuli to the animal and recording single unit responses to these stimuli. Amplitude and frequency modulated stimuli are particularly effective stimuli and appear to offer considerable promise for an analysis of integrative mechanisms in the auditory system.

The ionic basis for the receptor potentials produced by light in the Limulus eye is being studied. Preliminary results indicate that chloride ion is not involved in the receptor process and that light may increase the permeability of the receptor cell membrane to sodium.

Serial No.: NDB(I)-62 LNP/SC-934
1. Laboratory of Neurophysiology
2. Section on Spinal Cord
3. Bethesda Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Basic mechanisms of synaptic transmission

Principal Investigators: T.G. Smith, K.Frank, P.G. Nelson, R. Wuerker, R. Burke and F.Hiltz[1]

Other Investigators: M. Chapman, J.S. Bryan[2] and W. Rall[3]

Cooperating Units: 1/ Applied Physics Laboratory, Johns Hopkins University

2/ Section on Technical Development, NIMH-NINDB

3/ Office of Director of Intramural Research, NIAMD

Man Years:

Total:	4.3
Professional:	2.7
Other:	1.6

Project Description:

Objectives: The overall objective of the project is to elucidate the mechanisms of transmission of information between neurons, with emphasis on a comparative approach utilizing different preparations.

Important areas of study are: (1) the analysis of subthreshold spontaneous activity at synaptic junctions in the central nervous system and in peripheral neural structures; (2) the manner in which such spontaneous synaptic activity may determine cell excitability; (3) the localization of various synaptic contacts on axons, somata, and dendritic trees of neurons and the role of such localization in the integration of these synaptic connections; (4) the role that cell size or membrane resistance may play in determining excitability, and (5) the extent to which the electric fields occurring around firing neurons may influence the excitability of neighboring cells.

Major findings: Detailed computer analysis of spontaneous miniature synaptic potentials has been carried out in three cells and data collected from several more. Even under conditions tending to minimize spike activity in the spinal cord, the miniature potentials do not seem to be a random process and varying degrees of periodicity in their occurrence have been found. With graded degrees of input to the spinal cord evoked by muscle stretch, miniature PSPs appear to sum to the point of evoking spike activity in motoneurons.

The impedance change in motoneurons occurring during synaptic activity has been measured. Inhibitory postsynaptic potentials (IPSPs) invariably are accompanied by an impedance change, but the excitatory PSP (EPSP) is not accompanied by an impedance change in about half of the cells. While theoretical calculation by Wilfred Rall shows that this does not rule out the usual chemical theory of synaptic transmission, the finding does require considerable elaboration of the theory to include the dendritic location of the synaptic input. The data is also consistent with an electrical mechanism of synaptic transmission.

The possibility that different groups of synapses have different distribution in the dendrites has been tested by examining the manner in which PSPs add when elicited by stimulation of different afferent pathways. The results are too complex to be explained by any simple homogeneous distribution of synapses in the dendritic tree.

Cell size or overall cell resistance has been put forward by Elwood Henneman and coworkers as an important determinant of cell excitability and a direct test of this hypothesis, using intracellular techniques, has been attempted. The data confirm the hypothesis within the sampling limit imposed by the method.

A short latency increase of up to 30 percent in motoneuron excitability has been shown to accompany firing of adjacent motoneurons. This would not appear to be explainable on the basis of synaptic interaction and probably is a result of electrical interaction between cells.

Significance of research: The individual miniature PSPs appear to be the elementary units of communication between nerve cells. The quantitative analysis of these units appear feasible and should be helpful in understanding synaptically-induced spike generation in the central nervous system. It seems clear that the miniature potentials exhibit considerable

deviation from a model involving purely random processes.

Both the interaction of PSPs and the impedance measurements during synaptic transmission indicate that the dendritic location of synaptic input is of great importance in determining the effect of that input on a cell. It is hoped that a quantitative study of this factor in the motoneuron where a large number of definable inputs is available may be generalized to other situations where technical factors are not so favorable.

The question of electrical interaction between nerve cells has received much attention recently and the present experiments indicate that such interaction may occur in the mammalian central nervous system. Further experiments are necessary, however, to determine what functional importance, if any, this may have.

Proposed course of research: Further experiments on the miniature PSPs occurring in cat motoneurons in response to afferent stimulation are planned. Theoretical analysis of these potentials with respect to generation of spikes will be attempted.

Quantitative analysis is planned of the interaction of PSPs in terms of a model proposed by Wilfred Rall. This may permit greater precision in the localization of synapses on the cell dendrites than has heretofore been possible.

Part B Included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Hunt, C.C. and Nelson, P.G.: Structural and functional changes in the frog sympathetic ganglion following cutting of the presynaptic nerve fibres. J. Physiol. 177: 1-20, 1965.

Honors and Awards relating to this project: None

Serial No.: NDB(I)-58-LNP/SC-501
1. Laboratory of Neurophysiology
2. Section on Spinal Cord
3. Bethesda, Maryland
4. Continuation

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Generation of impulses in nerve cells

Principal Investigators: P.G. Nelson, B.O. Alving

Other Investigators: M.W. Chapman, A. Bak

Cooperating Units: None

Man Years:

Total:	2.9
Professional:	1.5
Other:	1.4

Project Description:

Objective: (1) To study the extent to which dendrites participate in spike generation in motoneurons; (2) to explore some properties of the nerve membrane which may affect the integrative function of the cell or its spontaneous activity.

Method employed: Cat motoneurons and snail and marine molluscan ganglion cells have been studied with intracellular electrodes. The effects of electrical currents, ions and drugs upon the nerve membranes on membrane resistance and voltage can be analyzed using voltage and current clamp techniques.

Major findings: Anomalous rectification, previously described in the giant cells of Aplysia by Kandel and Tauc has been found in snail ganglion cells and in cat motoneurons. Correlation of this property with behavior of the EPSP in cat motoneurons probably explains the heretofore anomalous behavior of the EPSP with hyperpolarization of the cell membrane.

A feature of the motoneuronal intracellular spike described by Granit and coworkers and termed delayed depolarization (DD) has been studied in relation to extracellular recordings. The DD appears to be attributable to spike activity in dendrites and is markedly affected by synaptic

input to motoneurons. Preliminary analysis of generator and spike potentials in Aplysia and snail ganglion cells indicates that relatively slow fluctuations in ionic permeability may underlie generator potentials and that the process of spike initiation may vary from cell to cell, depending on the manner in which the cells are activated.

Significance of research: Nerve cell dendrites are structures directly involved in central integrative processes and their passive and active electrical properties are therefore of importance in the consideration of interactions between cells. Anomalous rectification is a membrane property which can alter markedly the effectiveness of synaptic impingement on a cell.

Proposed course of research: The Aplysia giant neurons will be utilized for studying a number of aspects of nerve membrane behavior: 1) pacemaker activity is prominent in some of these cells and the effects of electrical currents in ionic environment and drugs on this activity will be carried out; 2) measurements of intracellular ionic concentration in various types of cells will be attempted; and 3) a rigorous study of the membrane effects of drugs under voltage and current clamp conditions can be made.

Part B Included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Nelson, P.G. and Frank, K.: Extracellular potential fields of single spinal motoneurons. J.Neurophysiol. 27:913-927, 1964.

Nelson, P.G. and Frank, K.: Orthodromically produced changes in motoneuronal extracellular fields. J.Neurophysiol. 27:928-941, 1964.

Honors and Awards relating to this project: None

Serial No.:NDB(I)-62-LNP/SC-973
1. Laboratory of Neurophysiology
2. Section on Spinal Cord
3. Bethesda, Maryland
4. Reactivated (NDB-NP/SC-10, 1962)

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Integrative mechanisms in the central auditory pathway

Principal Investigators: P.G. Nelson, S.D. Erulkar[1] and J.S. Bryan[2]

Other Investigators: R. Evans and M.W. Chapman

Cooperating Units: 1/ Department of Pharmacology, University of Pennsylvania

2/ Section on Technical Development, NINDB-NIMH

Man Years:

Total:	2.5
Professional:	1.3
Other:	1.2

Project Description:

Objective: To study patterns of unitary spike discharge in the central auditory pathway occurring in response to a variety of acoustic stimuli in an attempt to understand some of the mechanisms underlying the sensory discrimination performed by the auditory system.

Methods: Extracellular recordings have been made from the inferior colliculus of anesthetized cats. A voltage controlled beat frequency oscillator has been developed and, under the control of a LINC digital computer, can produce a variety of auditory stimuli. Tone pips, steady tones, and amplitude and frequency modulated tones have been used. The LINC computer has been programmed to analyze and display single unit responses during the course of experiments. Electronic models of the auditory system have been constructed in an attempt to simulate some of the firing patterns observed in the physiological experiments.

Major findings: Frequency modulated (FM) tones are very effective auditory stimuli and may evoke firing of a unit when steady tones or broad band noises do not. Very critical dependence of the magnitude and timing of the unit responses upon modulation depth or rate was seen. In order to understand the unitary responses to AM and FM stimuli it appeared that the unit's sensitivity to tones of different frequencies had to be considered in at least three ways: 1) On and Off response; 2) steady state and transient responses, and 3) excitatory and inhibitory responses.

Significance of research: Sensory coding in the auditory system involves complex synaptic interaction of such a nature that temporal characteristics of the stimulus strongly determine the response of the system. Qualitatively different effects may be produced by a given stimulus depending on the rate at which it is given or the temporal relationship between it and other stimuli. It is hoped that focusing on this aspect of the auditory system will lead to a much better understanding of the system and sensory coding in general.

Various response patterns seen experimentally have been simulated electronically. It is hoped that a rigorous analysis of the simulating system will be of use in forming testable hypotheses with regard to the mechanism of integration in the auditory system.

Proposed course of research: Extension of these studies are planned along two lines: 1) increase the variety and flexibility of the stimulus patterns; 2) study cell response at different levels of the auditory system in cochlear nucleus and medial geniculate body and, if possible, cochlear nerve and cortex.

Part B Included: No

Serial No.: NDB(I)-65-LNP/SC-1239
1. Laboratory of Neurophysiology
2. Section on Spinal Cord
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Photoreceptors in the Limulus eye

Principal Investigators: T.G. Smith, Jr.

Other Investigators: None

Cooperating Units: Section on Physiology, Ophthalmology Branch, NINDB (See NDB(I)-60-O/OPS-700c)

Department of Biology, Massachusetts Institute of Technology

Man Years:

Total:	0.5
Professional:	0.5
Other:	None

Project Description:

Objective and Methods: One of the main problems of interest in visual physiology is in ascertaining the mechanisms by which light energy is transduced into electrical energy by visual photoreceptors. Because of technical considerations, much of the fundamental work in this area has been done on the horseshoe crab (Limulus polyphemus). Previous experiments have demonstrated that light produces a depolarization (receptor potential) of the primary receptor cells (retinula cells) of the Limulus eye. Recently this potential has been shown to be a consequence of an increased conductance in the retinula cell membrane. Since such conductance changes are usually the manifestation of specific ionic permeability changes, the present experiments were designed to find out to which ion species the membrane becomes more permeable during stimulation by light.

Major findings: Replacement of extracellular chloride ions with sulfate ions produced no significant alterations in the retinula cell steady-state conductance or in its response to light stimulation. Replacement of sodium ion with Tris (hydroxy methyl) amino methane ion solution markedly increased

membrane steady-state conductance and essentially abolished the retinula cell's responsiveness to light.

Significance of research: At first glance, these results suggest: 1) that chloride ion participates insignificantly in retinula cell steady-state conductance and responsiveness to light, and 2) that the main effect of light is to increase the receptor cell's permeability to sodium ions. The increased steady-state conductance produced by replacing sodium with Tris, however, does not allow the second conclusion at present. The Tris may have produced "non-specific" and "toxic" alterations in the membrane and thereby eliminated its responsiveness to light.

Proposed course of research: As a test of the role of sodium permeability of retinula cell membrane during light stimulation and of the possible non-specific effects of Tris ions, sodium replacements by other cations, viz, choline and cesium, will be undertaken. Since these ions are chemically quite different from Tris and from one another, their non-specific effects would be expected to be different and perhaps negligible.

Part B Included: No

ANNUAL REPORT
July 1, 1964 through June 30, 1965
Laboratory of Biophysics, Intramural Research
National Institute of Neurological Diseases and Blindness

Robert E Taylor, Associate Chief

An understanding of the basic mechanisms involved in the operation of the nervous and neuromuscular systems of animals, including man, is one of the outstanding problems of our time which appears to be susceptible to attack by methods of investigation which are now available.

At the present time the activities of the members of the staff of the Laboratory of Biophysics are mostly directed toward contributing to an understanding of the underlying mechanisms involved in the initiation, propagation and termination of excitation in nerve and similar irritable structures. Without considering the items of growth, maintenance and repair some aspect of the functioning of a sensory receptor of the nervous system, such as the eye, and of the effector cells such as muscles and glands seems to be intimately associated with the effects that electrical potentials and chemical substances have on the ease with which ions cross the membranes of the cells. Scientists in the Laboratory of Biophysics are studying fundamental properties of those natural and artificial systems which lend themselves particularly well to studies of a basic physico-chemical nature. These studies involve the development of sophisticated electronic and mechanical devices, experimental studies of nerve membranes and artificial systems such as monomolecular films and metal solution interfaces and a considerable amount of thinking about the results and derivation of mathematical models with further studies of their properties.

Progress has been made toward solving some very old problems concerning the basis for the electrical activity in nerve cells. Earlier ideas of ion movement through the cell membrane with the electrical activity resulting from voltage dependent ion permeabilities appear to be essentially correct but only with the recently developed techniques of internal replacement of the contents of the nerve fiber of the squid with known solutions has it been possible to obtain precise information. Calcium has long been thought to be somehow intimately involved in the activity of nerve cell membranes and with the internal replacement of the cytoplasm of the nerve of the giant Chilean squid it has been found that with identical solutions on the inside and the outside of either sodium fluoride or potassium fluoride the nerve membrane shows no rectification or other interesting electrical properties but behaves like a simple negatively charged membrane which discriminates against anions and favors potassium over sodium by about ten to one. Further experiments along this line are in progress and it is expected that the results will provide detailed information about the precise role of calcium ions and interactions between sodium and potassium ions which are required in order to rationally construct a molecular model of the membrane.

Other experimental data has been acquired and analyzed concerning the effects of slowly changing processes. These are very difficult to study but are of importance in connection with repetitive activity, common in sensory systems and in the central nervous system. These studies of slow processes have not yet been undertaken using the combined techniques of voltage clamping and continuous perfusion of the fiber, a procedure which is necessary because of the unknown changes in internal concentrations accompanying applied currents of long duration.

It has been known for almost forty years that there is a significant resting ammonia production which increases with activity in nerve fibers and recently we have shown that the ammonium ion can pass through both the sodium and potassium channels to some extent. The Laboratory of Biophysics is not equipped at this time to investigate the possible metabolic relationships but the details of the degree to which ammonium and other ions such as lithium may be able to substitute for sodium or potassium should yield valuable clues as to the underlying molecular structures involved.

Further evidence which strengthens the ideas of a mosaic structure of the membrane has come from the comparison of the dielectric properties of the membranes studied in this laboratory with results of others on artificial thin films of lipid. The capacitance of the natural membrane is greater than the artificial lipid films and varies with frequency. It is suggested that the observed properties of the natural membrane may result from the addition to a basic lipid structure of islands of material having a high dielectric constant and large electrical losses.

Preliminary work has been started on the study of artificial systems and the application of newer physical techniques to natural membranes but this program will not be fully established for some time. Studies of adsorption of a number of cations to monomolecular films of phospholipids extracted from nerve tissue were done in collaboration with a group at the Massachusetts Institute of Technology. Much further work of this type is needed to compare with the emerging results of the studies of the interactions of these ions in the functioning of the nerve membrane.

A considerable amount of theoretical work on excitable membrane properties has been accomplished, much of it in conjunction with the electronic analog computer of the Laboratory of Biophysics and the digital computers at the NIH. Further investigation of the properties of the empirical equations of Hodgkin and Huxley describing movement of ions across membranes have been carried out in connection with the effect of temperature on threshold of excitation. A general statistical model has been developed for the kinetics of ion movements across nerve membranes. This model is based on the concept that the effect of the applied electric field is a dual one of opening gates or reversing dipoles in the membrane. This determines the percentage of existing channels which are open for the movement of ions which are then driven through by the combined electrical and osmotic forces. Without specifying the chemical nature of the "gates" this model will predict the kinetics of the movements of potassium ions and probably will subsume a number of more specific models which have been proposed with entirely arbitrary assumptions about the molecular nature of the mechanisms involved.

A simplified set of partial differential equations was developed in the Laboratory which retain all of the essential features of the Hodgkin-Huxley equations. These have been programmed for the LINC computer and a film has been made for a theoretical axon demonstrating the phenomena of threshold, propagation of impulses and other physiological phenomena of interest. This is a valuable contribution as a teaching aid and a research tool.

While the study of nerve nets is being given considerable attention by industrial and other laboratories at this time, it is felt that very few problems can be sufficiently well formulated to be accessible to attack by this laboratory with its present resources. However there is underway a highly profitable collaboration with a mathematician in the Computation and Data Processing Branch which is expected to yield a significant mathematical paper concerning the problem of how to interconnect a set of neurons so as to maximize the information storage capacity.

The Chief of the Laboratory has reviewed and extended thirty years of work on excitable membranes and concludes that the simple process of electro-diffusion cannot account for the behavior of the nerve membrane without adding subsidiary processes such as the potential dependent gates discussed above.

There have been a number of cases where individual members of the laboratory have been recognized by invitations to present their views. These have included the Ninth Bowditch Lecture of the American Physiological Society, a Gordon Conference on Biomathematics, lectures at Cornell University Medical College and Drew University, a work session of the Neurosciences Research Program in Boston, a symposium of the American Institute of Chemical Engineers and a conference in Miami on "Newer properties of perfused axons."

Serial No. NDB(I)-62 LB/CB 935
1. Laboratory of Biophysics
2. Section on Cellular Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Ionic Permeabilities of Excitable Membranes. Electrical Experiments and Analyses.

Principal Investigator: Taylor, Robert E

Other Investigators: Adelman, W.J., Jr.,(University of Maryland, Baltimore,Md.) and Guttman, R. (Brooklyn College, Brooklyn, New York)

Cooperating Unit: Marine Biological Laboratory, Woods Hole, Massachusetts

Man Years

Total:	0.5
Professional:	0.3
Other:	0.2

Project Description:

Objectives: The ionic current flow across the membrane of the squid giant axon has been measured, without the complications of excitation and propagation, after a sudden change of the membrane potential. The currents have been analyzed in terms of the membrane permeability to sodium and potassium ions. Much of classical nerve physiology is explained by these permeabilities which are themselves not understood. The long range objectives are the further interpretation of nerve function in terms of these fast ionic permeabilities and the elucidation of the structures and mechanisms by which the permeabilities are controlled.

Methods Employed: The original instrumentation of the potential control concept has been extensively modified and expanded in continuing work with the squid axon. The membrane potential is measured between micropipette internal and nearby external reversible electrodes and is maintained and changed as required by an electronic control system. This system computes, produces, and

measures the necessary flow of membrane current between axial and external axon electrodes to follow commanded rapid changes of the membrane potential. Considerable progress has been made in improving the reliability, speed and simplicity of the system.

Following developmental work in Bethesda using lobster axons, Doctor Guttman investigated the temperature characteristics of excitability of squid axons using the sucrose gap technique.

Major Findings: The independence principal relations appear to hold to a fairly good approximation for changes in external sodium concentrations. In addition the external sodium concentrations does not effect the potassium current. These results would imply that the electrical potential profile (or barrier height) through the membrane is not influenced by the space charges of the ions carrying the current. We feel that this is strong evidence that the diffusion equation as ordinarily employed does not apply to the squid axon membrane. It was found that the threshold temperature relations depend upon the pulse duration employed. The experimental results were compared with the theoretical predictions of Doctor FitzHugh.

Scientific Significance: A knowledge of the relations between concentration and current is of importance both for extending and improving the form of the empirical equations of Hodgkin and Huxley and for further investigations into the molecular mechanisms underlying the voltage dependent permeabilities responsible for the electrical activity.

Proposed Course of Project: Further experimental work will be combined with replacement of the contents of the axons with artificial solutions and investigation of the current voltage curves at very short times after a potential step.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Adelman, W. J., Jr., and Taylor, R. E.: Effects of replacement of external sodium chloride with sucrose on membrane currents of the squid giant axon. Biophys. J. 4: 451-463, Nov. 1964.

Kishimoto, U.: Current voltage relations in nitella. Jap. J. of Physio. 14: 515-527, Oct. 1964.

Honors and Awards relating to this project: None

Serial No. NDB(I)-62 LB/MA 936
1. Laboratory of Biophysics
2. Section on Mathematical Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Ionic Permeabilities of Nerve Membranes: Theoretical Investigations.

Principal Investigator: FitzHugh, R.

Other Investigators: Cole, K. S., and Lecar, H.

Cooperating Unit: Division of Research Services, NIH

Man Years:

Total:	1.8
Professional:	1.8
Other:	0.0

Project Description:

Objectives: To investigate the bases, consequences, and extensions of ionic permeability concepts by mathematical analysis and computation.

Methods Employed: Mathematical analysis, with particular emphasis on theories of nonlinear differential equations, stability and cable equations, has been used in conjunction with the electronic analog computer in this laboratory and the digital computers at the NIH

Major Findings: Manuscripts for three projects started last year are now in the final stages of revision, the investigation of the effect of temperature on threshold of the H&H (Hodgkin-Huxley) model, the chapter "Mathematical Models of Excitation and Propagation in Nerve," and the description of an electronic model of the neuron for demonstration purposes.

A general model has been developed for the kinetics of the specific ionic conductances in the nerve membrane. It is a statistical model based on the concept of the motion of charged particles under the influence of the

electric field across the membrane. When one or more charged particles occupy sites associated with a pore, that pore is blocked to the passage of current-carrying ions. The model can have as many conductance variables of state, of the activation and inactivation types of H&H, as needed to fit experimental data. Equations for the time course of the conductance changes are derived and appear to give a good fit to H&H's data on potassium currents, and possibly also on sodium, although the latter have not been analyzed in detail yet. The chemical nature of the charged particles, which could be either ions or dipoles, is not specified, but is accessible to experimental investigation of the effects of chemical agents on the time course of conductance changes.

One such model, in which the pores are blocked by rotating polar groups, has been studied in more detail. This model gives a qualitative explanation of the membrane conductance (including the negative resistance region) and conductance time constants as functions of membrane potential. Two related theoretical questions being studied are the behavior in strong electric fields of polar groups in molecular films, and electrical noise fluctuations in nerve as predicted by the various kinetic models.

The partial differential equations describing a continuous (unmyelinated) axon with a BVP membrane have been programmed for the LINC computer by Mr. James Bryan and Mr. William Sheriff of the Technical Development Section, NIMH. Solutions of the equations for various cases of stimulation through electrodes of the theoretical axon show the phenomena of threshold, refractoriness, mutual cancellation of colliding impulses, one-way and two-way propagation resulting from stimulating in the wake of a passing impulse, etc. Solutions are photographed on motion picture film frame by frame. A preliminary film has been made and shown informally. When a more complete film is obtained, it is expected to be a valuable teaching aid for physiology students. The method can also be used as a research tool to study such questions as the stability of the propagated impulse and decremental conduction.

One problem considerably removed from the other laboratory work arose by considering the possibility of a system of interconnected neurons as a nonlinear system

of many variables, having a high-dimensional phase divided into stability compartments. The mathematical problem of analyzing such models is very formidable, but as a first step it was proposed that a combinatorial study be made of the statistical distribution of the number of compartments of a system described merely by a phase space consisting of a finite set of state points. To each point there is assigned another state point at random as a successor, and the state of the whole system proceeds successively from point to point at successive instants of time. This model is multistable but completely random, i.e., has no preassigned structure at all. It provides a null reference system against which various structural assumptions of a neural net could be tested, the object being to find out how best to interconnect a set of neurons so as to maximize its information-storing capacity. This mathematically difficult initial problem was attacked by Mr. James Kiefer of the Computation and Data Processing Branch, DRS, who, with remarkable mathematical ingenuity, has succeeded in deriving an asymptotic expression for the average number of compartments as a function of the number of state points. Because of the difficulty of establishing even this preliminary result, the general problem will probably not be carried further in the near future, but it is expected to provide a significant mathematical paper by Mr. Kiefer.

The applicability of the theory of ion movements in electric and concentration gradients as begun by Planck to the squid axon membrane has been reviewed by Doctor Cole and appears in the April 1965 issue of Physiological Reviews. This review adds more and stronger reasons for thinking that the simple process of electro diffusion as analyzed to date is not a principal factor in the behavior of the squid axon membrane.

Scientific Significance: The concept and the measurements of the sodium and potassium ion movements across the squid axon membrane offer a highly specific and general approach to the factors underlying normal and pathological nervous processes which cannot as yet be investigated directly in higher animals and man. An analysis of the mathematical properties of the Hodgkin-Huxley equations makes possible a better understanding of their possible modifications and their limitations than is obtainable by a purely physical interpretation.

The extension of mathematical models of the nerve membrane to chemically nonspecific kinetic models is a logical step beyond the largely empirical model of Hodgkin and Huxley, and leads the way to more specific molecular models. Experimental data on which to base molecular models is lacking, but kinetic models may help to plan experiments for obtaining such data.

The contrasts between calculations and the array of experimental facts give a basis to conclude that the simple process of electro diffusion is not a principal factor in the behavior of the squid axon membrane.

Proposed Course of Project: Further theoretical study of the propagation of impulses, their stability, and relationship to decremental conduction, appears to be a promising direction for research. However, the mathematical difficulties are greater than for the nonpropagated cases, and progress may be slower.

The effect of varying a number of the parameters of the H&H equations on the stability of the resting state, upon which depends the presence or absence of spontaneous trains of impulses, should be worked out systematically and published.

Enough new experimental information has been accumulated, especially with respect to more powerful ionic currents, the nonlinearities in the ionic currents, and the production of finite trains of impulses involving long-term accommodation, to justify an attempt to modify the H&H equations so as to embody these results.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Cole, K. S.: Theory, experiments and the nerve impulse. In Waterman, T. H., and Marowitz, H. J. (Eds.): Theoretical and Mathematical Biology. New York, N. Y., Blaisdell Publishing Co., 1965, pp. 136-171.

_____: Electra diffusion models for the membrane of the squid giant axon. Physio. Rev. 45: ____ - ____, April 1965.

FitzHugh, R., and Cole, K. S.: Theoretical potassium loss from squid axons as a function of temperature. Biophys. J. 4: 357-265, July 1964.

Honors and Awards relating to this project: None

Serial No. NDB(I)-62 LB/ME 937
1. Laboratory of Biophysics
2. Section on Membrane Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Ionic Permeabilities of Nerve Membranes. The Lobster Giant Axon.

Principal Investigators: Taylor, R. E and Guttman, R., (Special Consultant)

Other Investigators: Goldman, D. E., and Binstock, L.

Cooperating Unit: Naval Medical Research Institute

Man Years:
None

Project Description:

Objectives: The work on giant axons of the lobster will be included in other project descriptions. The direct collaboration has been discontinued and this project is terminated.

Serial No. NDB(I)-62 LB/ME 938
1. Laboratory of Biophysics
2. Section on Membrane Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Alternating Current Bridge Measurement of Squid Axon Membrane Dielectric Properties.

Principal Investigators: Taylor, R. E

Other Investigators: Chandler, W. K. (Cambridge University, Cambridge, England)

Cooperating Unit: Marine Biological Laboratory, Woods Hole, Massachusetts

Man Years

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives: It would seem reasonable that the regions of membrane involved in the passage of the ionic current is physically distinct from that part responsible for the measured membrane capacity, and thus that a knowledge of the electrical properties of this region is essential to any detailed understanding of the membrane structure. The pioneering work of Cole and Curtis some twenty years ago has stood the test of time, but this work has not been repeated until now and several gaps remain; the effects of temperature, effects of long continued membrane potential changes, possible drug and ion effects and others. No reasonable physical model has been proposed to explain the frequency dependence of the measured dielectric properties.

Methods Employed: A precision Schering bridge was developed for alterating current measurements from 10 to 70 kilocycles. An internal platinum wire electrode was used with cell and other apparatus previously described for voltage clamp measurements on the squid giant axon.

Major Findings: Further analytical work using the membrane impedance data collected with Doctor Chandler has

resulted in further and more definite conclusions about the nature of the dielectric of the squid axon membrane. Because any resistance in series with a lossy dielectric is unknowable from electrical measurements an attempt was made to find a best fit for the data assuming that the dielectric had a single relaxation time constant, taking the series resistance as a parameter to be determined. The resulting fits were not perfect, but indicate that there is possibly a small range of time constants. On this assumption the values for the squid axon membrane appear to be approximately 0.86 microfarads per cm^2 at low and 0.62 at high frequencies, at 6°C with a relaxation time constant corresponding to a characteristic frequency of about 28 kilocycles. At 17° both values of capacitance increased by about 0.04 microfarads per cm^2 with little change in the characteristic frequency.

Scientific Significance: Recent measurements by a group in England yield a value for stable bilipid layers of about 0.4 microfarads per cm^2, independent of frequency. It thus appears that if the membrane is fundamentally built up on a bilipid basis there must be islands of higher dielectric constant, lossy material. It is a tempting possibility that these islands are responsible for the complex ion permeability properties of the natural membranes.

Proposed Course of Project: Further measurements of impedance are required. The recent development of the coaxial internal electrode for the squid axon in this laboratory should make such measurements feasible. It is also important to know the temperature dependence of the capacity of artificial bilip layers. On the lossy patch hypothesis this might give a basis for determining the relative areas of bilipid layer and lossy patch.

Part B included No

Serial No. NDB(I)-62 LB/CB 939
1. Laboratory of Biophysics
2. Section on Cellular Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Ionic Permeabilities of the Squid Giant Axon Membrane. Electrical Experiments and Analyses with Alterations of Environments.

Principal Investigators: Ehrenstein, G.M., Gilbert, D.L., and Rojas, E. (Visiting Associate)

Other Investigators: Binstock, L., Guttman, R. (Brooklyn College), Adelman, W. J., Jr. (University of Maryland, Baltimore, Md.) and Gerschman, R. (Universidad de Buenos Aires, Buenos Aires, Argentina)

Cooperating Unit: Marine Biological Laboratory, Woods Hole, Massachusetts. Estacion de Biologia Marina, Vina del Mar, Chile.

Man Years

Total:	4.2
Professional:	2.2
Other:	2.0

Project Description:

Objectives: The ionic current flow across the membrane of the squid giant axon has been measured, without the complications of excitation and propagation, after a sudden change of the membrane potential. The currents have been analyzed in terms of the membrane permeability to sodium and potassium ions. Much of classical nerve physiology is explained by these permeabilities which are themselves not understood. The long-range objectives are the further interpretations of nerve function in terms of these fast ionic permeabilities and the elucidation of the structures and mechanisms by which the permeabilities are controlled.

Methods Employed: The standard voltage clamp technique on squid nerve was used in these experiments. In some experiments, the technique was employed on internally perfused axons using the method of Adelman and Gilbert.

In the course of conducting single salt experiments new methods for continuous perfusion of the axons of the large Chilean squid were developed. These axons are large enough so that in addition to the presence of the infusion and suction pipettes the axons contained an axial current wire and a longitudinally inserted voltage measuring pipette for voltage clamping.

In another set of experiments, Dr. Eduardo Rojas collaborated with Dr. M. Canessa-Fischer of the University of Chile to combine continuous perfusion of these large axons with measurements of the movements of radioactive Ca^{++}, Na^{+}, K^{+}, Rb^{+}, and Cs^{+}.

Major Findings: The effect of replacing external sodium with potassium on the potassium currents of isolated intact squid axons was investigated in Woods Hole and Chile. These experiments indicate a potassium inactivation process similar to that of sodium but with a time constant in the order of hundreds of milliseconds. Another slower potassium inactivation process was observed with a time constant of the order of seconds.

Experiments were performed to test the validity of the independence principle for potassium ion currents. It was found that at the potential produced by the high external potassium concentration the currents were much smaller than predicted by the independent principle. When the slow inactivation was removed by hyperpolarization they were still smaller (95% in 40 mM, 80% in 100 mM and 60% in 440 mM) than predicted from the normal 10 mM external K.

The steady state current voltage relation of axons immersed in external isosmotic potassium chloride in the presence of 10 mM calcium and 50 mM magnesium shows a negative resistance region following hyperpolarization. With variation in external divalent ion concentration shifts occurred on the voltage axis as seen in normal potassium but the absolute values were different. This result suggests strongly that there is a potassium-calcium

antagonism in the mechanism controlling potassium conductance.

In the presence of only NaF or KF inside and outside of the axons of Docidicus gigas in Chile no time dependent permeability changes following step changes in membrane potential and no sharp dependence of permeability on potential were observed.

The results of Tasaki were confirmed that the presence of internal chloride blocks action potential production and that this block is quickly relieved by flouride ions.

The data accumulated on radioactive tracer movements is being analyzed.

Scientific Significance: Contemporary molecular models of the behavior of nerve membranes are conceived in darkness. The findings reported here are urgently needed and thus far rather incomplete.

Proposed Course of Project: Only by continued and extensive investigation of the effects of changes in internal and external ion concentrations will the evidence be forthcoming which can provide the basis of a rational attack on the problem of molecular mechanisms in the membrane. These experiments will be conducted on the only available material which is suitable, vis., the axons of the squid obtained in Woods Hole, Massachusetts and in Chile.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Adelman, W. J., Jr., and Gilbert, D. L.: Internally perfused squid axons studied under voltage clamp conditions. J. Cell. and Comp. Physiol. 64: 423-428.

Honors and Awards relating to this project: None

Serial No. NDB(I)-62 LB/I 940
1. Laboratory of Biophysics
2. Section on Instrumentation
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Ionic Permeabilities of the Squid Giant Axon. Effects of Chemical Agents.

Principal Investigators: Binstock, L. and Lecar, H.

Other Investigators: Armstrong, C. (University College London)

Cooperating Unit: Marine Biological Laboratory, Woods Hole, Massachusetts. Estacion de Biologia Marina, Vina del Mar, Chile.

Man Years
Total: 1.0
Professional: 1.0
Other: 0

Project Description:

Objectives: The voltage clamp technique allows measurements of ionic current flows across nerve membrane as a function of voltage and time. These parameters have been combined into a set of empirical equations capable of expressing nerve membrane activity, but the physical meaning of the equations is unknown. It is hoped that the use of various chemicals known to affect nervous activity may contribute to understanding the physical mechanisms of the membrane. Irrespective of the chemical properties of the substance, this may permit dissection of the lumped parameters of the equations as well as a correlation of known chemical properties with the observed effects.

Methods Employed: The general aspects of the voltage clamp technique employed were described previously. The penetrating microelectrode has been eliminated because there is no way to compensate for the distortions which are produced at short times following the application of a potential step. The

present internal coaxial electrode is a practical version of the first hand-made electrodes of Armstrong and Binstock. With further improvements, particularly with solid state preamplifiers the response time of the system has been further shortened.

Major Findings: Detailed, quantitative studies were made with Lithium substituted for sodium. Both magnitudes and time constants of the permeability changes for Lithium are the same as for Sodium.

Substitution of Ammonium ions for external Sodium results in an enhancement of the steady state outward current under voltage clamp. Early inward currents were obtained with a large hyperpolarizing prepulse or holding potential. Clearly these currents cannot be carried by sodium ions which are not there. Post Anodal action potentials could be produced which were smaller than normal.

A manuscript has been completed and accepted for publication on the effects of internally injected Tetraethylammonium ions.

Scientific Significance: That lithium is such a good substitute for sodium suggests that the mechanisms in the membrane responsible for ion selectivity are separate and distinct from those responsible for the kinetics of the ion permeability changes with potential.

The results with ammonium ions indicate that the variable results found with different kinds of nerves with this substance are probably related to its being a partial substitute for both sodium and potassium ions. The implications of these findings in terms of detailed mechanisms is still obscure but should be the raw material for future suggestions. One important question involved is whether the sodium and potassium ions cross the membrane through separate channels.

Proposed Course of Action: Further experimental work is planned to determine the details of the effects of ammonium ion. Of particular interest are the effects of temperature and pH and the use of TEA to block the potassium component of the currents.

Part B Included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Armstrong, C. M., and Binstock, L.: The effects of several alcohols on the properties of the squid giant axon. J. Gen. Physiol. 48: 265-277, November 1964.

Armstrong, C. M., and Binstock, L.: Anomalous Rectification in the Squid Giant Axon Injected with Tetraethylammonium Chloride. J. Gen. Physiol., May 1965 (in press).

Honors and Awards relating to this project: None

Serial No. NDB(I)-65 LB/ME 1240
1. Laboratory of Biophysics
2. Section on Membrane Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Molecular Biophysics - Physical Properties of Simple Membrane-like Systems.

Principal Investigators: Ehrenstein, G., Lecar, H., and Rojas, E.

Other Investigators: Lettvin, J. Y. and Pickard, W. F. (MIT)

Cooperating Unit: MIT

Man Years

Total:	0.2
Professional:	0.2
Other:	0

Project Description:

Objectives: To study the ionic permeabilities, electrical properties and molecular structure of thin films, bulk lipid, liquid crystals and other related physical systems. To examine related molecular properties of excitable membranes by newer physical techniques.

Methods Employed: Properties of lipid monolayers at an air-water interface and of synthetic bimolecular leaflets between two water phases are determined in various ways, e.g., water permeability, selective ion adsorption and structural changes in intense electric fields. Thermal noise fluctuations can be measured in the intact and perfused giant axon of the squid or single myelinated nodes using electrophysiological techniques.

Major Findings:

(a) Adsorption studies on phospholipid: These experiments were performed at the Massachusetts Institute of Technology and a manuscript of a

paper by E. Rojas, J. Y. Lettvin, and W. F. Pickard has been prepared. Tracer studies were made of the selective adsorption of cations on phospholipid monolayers. An exchange of ions between the polar groups of the phospholipid and the hypophase was found. The separation factor between La^{+++} and Ca^{++} was about 10^{-3} and and between Ca^{++} and any of the following cations: Li^{+}, Na^{+}, K^{+}, Rb^{+}, Cs^{+}, was 5×10^{-3}.

(b) Thermal noise in the squid axon: Calculations and fabrication of test electrodes have been done in collaboration with Dr. W. A. Hagins of the Laboratory of Physical Biology, NIAMD.

Scientific Significance: In order to understand the workings of excitable membranes, the physiological data gathered mainly by electrical measurements must be supplemented by some knowledge of the underlying molecular processes. The monolayer studies have shown the explicit ionic selectivities of a simple system which is often thought to have aspects of the membrane structure. The noise studies may lead to a knowledge of the density of conducting sites on the nerve surface.

Future Course of Project: In addition to further studies on monomolecular and bimolecular films and studies of thermal noise fluctuations in natural and artificial membranes, feasibility studies are being conducted concerning the possible use of newer physical techniques such as (1) low energy electron diffraction as a tool for studying surface ordering, (2) microwave dielectric relaxation measurements for studying bound water in polymer solutions and monolayers and (3) conductance measurements on organic semiconductors in relation to the possible role of electronic conducting mechanisms in membranes.

Part B included No

ANNUAL REPORT
July 1, 1964 through June 30, 1965
Laboratory of Neurochemistry, Intramural Research,
National Institute of Neurological Diseases and Blindness

Dr. D. B. Tower, Chief

As a result of unanticipated shifts in training schedules, all but one of the Laboratory's complement of research associates departed early in the year. Thus, this has been one of the "quieter" years for the Laboratory. All will be back to normal at the beginning of the fiscal year 1966, when a full complement of research associates, as well as summer "students", report in. The permanent staff was strenghtened by the addition of a skilled organic chemist to the Lipid Chemistry Section, where already his contributions to investigations of sphingoglycolipid metabolism are proving most helpful. The long-planned changes in Laboratory office personnel have now been most satisfactorily accomplished. Two staff members have carried out important research assignments abroad in Buenos Aires and Tel Aviv respectively, and active collaboration with numerous intramural and extramural colleagues has continued to characterize all projects of this Laboratory. Specific references to such collaborations will be found in the individual project reports. Here it is appropriate again to acknowledge these mutually beneficial associations and the research progress stemming therefrom and to look forward to fruitful continuations. The Laboratory received the first detailed review of its programs by the NINDB Board of Scientific Counselors since the present organization of the Laboratory four years ago. Their comments and general endorsement were most gratifying.

The general direction of research in the Laboratory of Neurochemistry, as represented by the attached individual project reports, continues unchanged. The major emphasis remains in the so-called basic realm but upon problems with clear, eventual clinical applications. As in previous reports, this emphasis may be most simply summarized as one focussing upon the nature of neural membranes and mechanisms of bioelectrogenesis. In essence what is under study is interactions upon surfaces and at interfaces between sets of solutions of differing characteristics and metabolic potentialities. The interposition of the interfaces with their resultant surfaces is a direct consequence of membranes surrounding and subdividing the cell. The extremists would on the one hand consider these as no more than fluid-fluid interfaces or on the other as rigid barriers; the truth apparently lying about midway between. For the nervous system membranes are paramount since it is upon and across them that the functional attributes so characteristic of neuronal and neural activity occur. It becomes increasingly obvious that if we are to elucidate the details of such functional mechanisms and if we are to understand the ultimate significance of experimental or clinical dysfunctions, we must know intimately the compositional and structural organizations of these membranes and how these conformations relate to metabolic and physiological events associated therewith.

Few, if any, satisfactory approaches are available or potentially feasible for direct study of any such membranes pure and in isolation. Not only

is the nervous system one of the most heterocellular and complex tissues of the body, but it is apparent neurophysiologically that the nerve cell membranes exhibit a complex patchwork of independent but coordinated or ordered events taking place thereon. Thus, what one might consider, if obtainable, a morphologically pure membrane preparation is likely both biochemically and physiologically to be highly "impure", highly complex and heterofunctional. It is for these reasons that the scope of projects and approaches in this Laboratory are so broad and superficially not always so obviously interrelated. And in no sense are the various projects programmed or directed purposively to a single end, but only in retrospect can they be seen to have gravitated by mutual but independent confluence toward a common general goal.

At this stage the various projects are distributed among four or five aspects of the general problem, ranging from studies on "crude" membrane composition and artificial reconstitutions thereof, through investigations of lipid and protein components of membranes, to evaluation of fluid and electrolyte passage or transport across them and elucidation of the detailed mechanisms involved. The extensive studies by the Section on Lipid Chemistry on the sphingoglycolipids are this year particularly noteworthy. These molecules are essential constituents of various neural membranes (e.g., cerebrosides in myelin sheats; gangliosides in nerve cell membranes). Current work not only is yielding detailed information about the biosynthesis and turnover of these compounds but also rapidly clarifying the nature of various disorders associated with faulty metabolism of the compounds. Thus, we have seen this year the pin-pointing of the defect in cerebroside metabolism in Gaucher's disease, viz., a lack of glucocerebroside-cleaving enzyme. The techniques developed here are now being applied to the analogous conditions of Niemann-Pick's disease and Tay-Sach's disease. Additional relevance to demyelinating disorders is at once obvious.

Projects in the Sections on Physiology and Metabolism and on Proteins and Amino Acids are closely complementary to the foregoing as the lipoprotein and protein components of membranes continue to be investigated. It is already evident from studies on artifically reconstituted membranes and on preparations of "receptor" molecules that proteins as well as the lipid bilayers are essential to confer upon any membrane-like multimolecular complex the attributes known to be characteristic of natural membranes. Hence, the data from these projects may be expected to provide indications of the types or characteristics of proteins most appropriate in functional terms and to suggest how protein and lipid components are likely to be associated within membrane conformations. Such studies are both highly significant for our further understanding and at the same time most frustrating because of the inherent complexities of membranes and current shortcomings of our investigative techniques.

At the stages of ever increasing complexity are other projects in the Sections on Enzyme Chemistry and on Proteins and Amino Acids, both dealing with cation transport across neural membranes. In the former Section, the nature of the molecular assemblage represented by "transport ATPase" is gradually being unravelled. This continues to be a most significant and

fruitful study. In the latter Section, the problems of tissue and cellular compartmentation of cerebral fluids and electrolytes represent the most complex level of investigation short of in vivo studies. The recent interpretations suggested by studies on neonatal and developing brain seem to promise extensive clarification of the rather confusing situation in mature cerebral cortex, such that a more meaningful approach to study of cation fluxes in whole cell, incubated slice preparations will now be possible.

In this portion of the annual report for the Laboratory of Neurochmistry, I have repeatedly stressed the complexities involved. This is both deliberate and in no sense an apologia. What is implied is perhaps best expressed by quoting a recent opinion on this general point by Prof. P. A. Srere, who wrote: "(It has been) said that remarkable scientific achievements have come from 'the methods of simplifying thought by stressing one aspect only of the facts'. None the less, in biological investigations we must deal with systems that are exceptionally complex and we run the danger of choosing model systems that are too simple to advance our understanding. To understand cellular metabolism we must continually search for model systems the complexity of which approaches as closely as possible that of the cell. The models should have been sufficiently examined to enable us to make some simple predictions and explanations of the more complex cellular systems." (Nature 205: 770, 1965).

These views are worth bearing in mind when one becomes impatient over rates of progress and when considering the immediate future of on-going projects. The general directions of future developments have been indicated in the foregoing discussion of Laboratory projects and objectives. A specific example emphasizes the viewpoint just cited. We can now reasonably expect that the metabolic derangements in most, if not all, of the lipidoses will shortly be susceptible of detailed explanation. Thus, in the case of Gaucher's disease we may now write in summary form:

A. (1) Acyl-CoA + serine ---> sphingosine
Sphingosine + UDP-galactose ---> psychosine
Psychosine + acyl-CoA --> cerebroside (gal)
(2) Cerebroside (gal) --> ceramide + galactose

B. (3) Erythrocyte stroma --> globoside (=cerebroside (glu)-gal-gal-N-ac-gal-NH_2)

(4) a. Globoside --> cerebroside (glu)-gal-gal + N-ac-gal-NH_2

b. Cerebroside (glu)-gal-gal -> -> cerebroside (glu) + 2 galactose

c. Cerebroside (glu) ---> ceramide + glucose

The biosynthesis (A.1) and catabolism (A.2) of galactocerebroside by normal subjects and patients with Gaucher's disease are evidently the same. The degradation of globoside, arising from aging and disposal of erythrocytes (B) also appears to be similar until reaction (4 c.) is reached, where in the Gaucher spleen, the enzyme (*) degrading glucocerebroside is deficient or missing, with consequent accumulation of glucocerebroside. A specific therapeutic approach to Gaucher's disease would thus involve either replacement of the missing enzyme (*) in reaction (4 c.) or inhibition of preceding

reactions (4 a or b) to prevent glucocerebroside production. Since we cannot yet "replace" missing enzymes in such circumstances, we are left with the latter alternative, which could conceivably be accomplished. But the consequences of such an approach must be clearly appreciated. Erythrocyte degradation and hence globoside production (reaction B.3) is a necessary step in erythrocyte turnover. Blockage of reactions (4 a) or (4 b) would lead to accumulation of globoside or its immediate derivatives and in effect substitute another for Gaucher's disease. In fact Nature has already done the experiment, in which the enzyme for reaction (4 a) is missing, providing us with Fabry's disease. Thus, even though the metabolic complexities associated with the deranged metabolism in the Gaucher spleen have been clarified, the complexities of applying this knowledge to clinical and therapeutic approaches persist.

One could adopt a pessimistic point of view and admit discouragement at ever really solving such problems. Yet it is precisely the knowledge and appreciation of our areas of ignorance that will in time provide the ultimate answers which we seek. In this sense, the projects of this Laboratory are basically concerned with defining these areas of ignorance and reducing them to manageable proportions. Short of serendipidity, this would seem to offer the only reasonable and logical routes for fruitful progress.

Serial No. NDB(I)-61 LNC/EC 813
1. Neurochemistry
2. Enzyme Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Enzymological Aspects of Neural Function

Principal Investigator: Dr. R. W. Albers

Other Investigators: Mr. G. J. Koval and Dr. S. Fahn

Cooperating Units: Dr. L. Guth, Section on Experimental Neurology, LNS, Dr. E. de Robertis, Instituto de Anatomia General, Universidad de Buenos Aires

Man Years (computed for the 12 month period)

Total:	4.6
Professional:	3.0
Other:	1.6

Project Description

Objectives: To assess the functional roles and interrelations of enzyme systems which characterize neural tissue.

Methods: See 1962 report.

Major Findings: (1) A detailed study of the properties of the microsomal adenosine triphosphatase from electric organ of Electrophorus electricus has led to information about the interaction of sodium and magnesium ions in the phosphorylation of the microsomes by ATP. Sodium ions had previously been reported to have little influence in this part of the reaction. However, we have been able to demonstrate a large effect of Na^+ on ATP-ADP transphosphorylation either (a) after treatment of the microsomes with N-ethyl maleimide or (b) in the presence of low Mg^{++} concentrations. The parameters of this effect have received careful study. The results provide further requirements which must be met by a hypothetical sodium ion pump employing this ATPase reaction.

Other workers have reported a K^+-activated p-nitrophenyl-phosphatase associated with the so-called microsome fraction of various tissues. We have found this enzyme activity to be high in electric organ microsomes; this supported the possibility that ATP and p-nitrophenylphosphate hydrolysis might be catalysed

Part A continued

by the same enzyme.

We have used two approaches in testing this hypothesis: (a) a careful comparison of the alkali metal activation characteristic of the two reactions in electric organ microsomes; (b) an investigation of the subcellular distribution of the two enzymes in brain tissue (in collaboration with Drs. deRobertis and Rodriguez de Lores Arnaiz at the University of Buenos Aires). Both studies support the conclusion that the enzymes are distinct entities although both appear to be constituents of cell membranes.

(2) The studies on the trophic aspects of the nerve muscle relationship have continued in collaboration with Dr. Guth. Details will be covered in his report. (Serial No. NDB(I)-63 LNS/EN 1054).

Significance: The Na^+-dependent ATPase is an enzyme closely associated with the cell membrane. The bulk of physiological evidence at present links this enzyme with the process of pumping Na^+ ions from the internal to the external surface of the cell membrane. The pharmacological actions of the cardiac glycosides are thought to be a result of their action on this enzyme. The enzyme preparation from Electrophorus electricus is to date the most active preparation which has been studied. The indications are that it is the most experimentally accessible material for study of the molecular transformations involved in ion transport.

Proposed Course: Projected studies of the "transport ATPase" include efforts to further test the hypothesis that the microsomal phosphorylation, transphosphorylation and hydrolase activities are manifestations of the catalytic activity of a single molecular assemblage. Several new technical approaches need to be developed to deal with highly labile and rapidly formed enzyme intermediates. In addition, a major goal is the identification of the type of chemical bonds formed in each stage of the ATPase reaction. A general investigation of the chemical composition of the electric organ microsomal fraction is planned as a guide to this endeavor.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B:

Publications:

1. Embree, L. J., and Albers, R. W.: Succinic semialdehyde dehydrogenase from human brain. Biochem. Pharm., 13: 1209-1217 (1964).

2. Fahn, S., Albers, R. W. and Koval, G. J.: Electrophorus adenosine triphosphase: Sodium-activated exchange after N-ethyl maleimide treatment. Science, 145:283-284 (1964).

3. Guth, L., Albers, R. W. and Brown, W. C.: Quantitative changes in cholinesterase activity of denervated muscle fibers and sole plates. Exp. Neurol., 10: 236-250 (1964).

4. Fahn, S., Albers, R. W., Koval, G. J.: Thin-layer chromatography for the separation of nucleotides. Anal. Biochem., 10:468-471 (1965).

5. Albers, R. W., Arnaiz, G. R. de Lores, and de Robertis, E. Sodium-potassium-activated ATPase and potassium-activated p-nitrophenylphosphatase: a comparison of their subcellular localization in rat brain. Proc. Nat. Acad. Sci., 53: 557-564 (1965).

Serial No. NDB(I)-61 LNC/LC 815
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Metabolism of Complex Lipids of Nervous Tissue

Principal Investigator: Dr. R. O. Brady

Other Investigators: Drs. J. N. Kanfer and A. Gal, Mr. R. M. Bradley, and Mr. O. Young

Cooperating Units: Dr. David Shapiro, Dept. of Organic Chemistry, Weizmann Institute of Science, Rehovoth, Israel, Dr. R. O. Scow, Section on Endocrinology, LNE, NIAMD

Man Years: (Computed for the 12 month period)

Total:	4.5
Professional:	3.2
Other:	1.3

Project Description:

Objectives: (1) To elucidate the biosynthetic pathways for the formation of long chain fatty acids, carebrosides and gangliosides; (2) to study the control mechanisms which regulate these processes; and (3) to study the metabolic fate of complex lipids such as cerebrosides, sphingomyelin and gangliosides in normal and lipodystrophic disease states.

Methods: Glucocerebroside labeled with ^{14}C in either the glucose or fatty acid portion of the molecule has been synthesized with the cooperation of Dr. David Shapiro. ^{14}C-labeled sphingomyelin and glucopsychosine are being simiarly prepared. The metabolism of these labeled materials will be studied in order to investigate the explicit metabolic defects in various sphingolipidoses such as Gaucher's and Niemann-Pick's disease.

Major Findings: (1) With the use of glucose-^{14}C-cerebroside, we have been able to demonstrate the presence of an enzyme in spleen which catalyzes the decomposition of this compound. Optimal conditions for the activity of this enzyme in normal human spleen and the sequence of the catabolic reactions involved have been demon-

Part A continued

strated. An examination was made of the level of this enzyme in splenic tissue from patients with Gaucher's disease. The nature of the metabolic error in Gaucher's patients has been identified as a specific deficiency of this glucocerebroside cleaving enzyme.
(2) For over 15 years it has been known that fatty acid synthesis in tissues of fasted and diabetic animals is drastically impaired. We have recently succeeded in pin-pointing the limiting and control reactions involved in this biosynthetic regulator phenomenon. Specifically this is related to availability of $NADPH_2$ from Glucose 6-P oxidation and the effects of circulating glucocorticoids on levels of activity of the enzymes involved. With this knowledge it has been possible to restore fatty acid synthesis to normal levels in these conditions with Dexamethasone.
(3) The activity of galactose oxidase has been investigated using a number of complex sphingolipids as substrates. This enzyme catalyzes the oxidation of bound, as well as free, galactose molecules. Since cerebrosides (of the myelin sheath) and gangliosides both contain galactose, this enzyme was employed as an adjunct to chemical studies of the structure of cerebrosides and gangliosides. It was observed that monosialoganglioside and asialoganglioside are equally active as substrates, but Tay-Sachs' ganglioside is only about one-fifth as active.
(4) We have devised an improved procedure for the separation of complex sphingoglycolipids on thin layer chromatography which permits resolution of all of the presently known compounds of this nature. We have also required a procedure to quantitatively determine long chain fatty acid amides which occur in the sphingolipids. A facile satisfactory method for these compounds has been accomplished in this laboratory.

Significance: The demonstration of a definitive enzymatic deficiency in Gaucher's disease has been accomplished. This finding pinpoints the metabolic error in this condition and is a prerequisite for the undertaking of a rational therapeutic approach to alleviate this condition. Analogous studies are underway in attempts to discover the nature of the metabolic deficiencies in Niemann-Pick's and Tay-Sachs' diseases.
The understanding of the factors regulating fatty acid synthesis is of fundamental importance in overall metabolism and particularly with regard to such conditions as diabetes, arteriosclerosis and obesity.

Proposed Course: The enzymes which catalyze the catabolism of glucocerebroside and galactocerebroside will be characterized and their distribution and properties investigated. Similar studies utilizing labeled sphingomyelin will be undertaken. Galactose-

Part A continued

^{14}C-Cerebroside has been synthesized in this laboratory. It will be used for metabolic studies in demylinating conditions. The formation and metabolism of sulfatides will be explored with the labeled galactocerebroside with the hope of elucidating the metabolic disturbance in metachromatic leukodystrophy which is characterized by the accumulation of sulfatide in various tissue.

Part B included: Yes

PHS-NIH
Individual Project Report
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Part B

Publications:

1. Brady, R. O., Kanfer, J., and Shapiro, D.: The metabolism of glucocerebrosides. I. Purification and properties of a glucocerebroside-cleaving enzyme from spleen. J. Biol. Chem., 204; 39-43 (1965).

2. Brady, R. O., Kanfer, J., and Shapiro, D.: The metabolism of glucocerebrosides. II. Evidence of an enzymatic deficiency in Gaucher's disease. Biochem. Biophys. Res. Comm., 18: 221-225 (1965).

3. Brady, R. O., Biosynthesis of Glycolipids. In Dawson, R.M.C. and Rhodes, D. N. (Eds). Metabolism and Physiological Significance of Lipids. John Wiley and Sons, Ltd., London, 1964, pp. 95-109.

4. Bradley, R. M., and Kanfer, J. N.: The action of galactose oxidase on several sphingoglycolipids. Biochim. Biophys. Acta, 84: 210-212 (1964).

5. Brady, R. O., and Trams, E. G.: The chemistry of lipids. Annual Review of Biochemistry, 33: 75-100 (1964).

6. Brady, R. O., Scow, R. O., Urgoiti, E., and Bradley, R. M.: The effect of pancreatectomy on fatty acid synthesis in liver of fasted rats. Biochim. Biophys. Acta, in press.

7. Young, O., and Kanfer, J. N.: Improved separation of sphingolipids by thin-layer chromatography. J. Chromatography, in press.

8. Bradley, R. M.: An improved method for the determination of long chain fatty acid amides. Biochim. Biophys. Acta, in press

Serial No. NDB(I)-61 LNC/LC 817
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Immunochemical Studies in Multiple Sclerosis

Principal Investigators: Drs. R. O. Brady and J. N. Kanfer

Cooperating Units: Dr. John E. Somers, Division of Neurology, University of Missouri Medical Center, Columbia, Missouri

Man Years: (Computed for the 12 month period)

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: To determine whether auto-immune phenomena participate in the pathogenesis of neurological diseases.

Methods: Examinations were made of the sera from patients with multiple sclerosis and animals with experimental allergic or viral encephalomyelitis for the presence of antibodies to gangliosides, asialogangliosides, myelin sheath lipids, etc. Animals were immunized by several procedures with various ganglioside preparations.

Major Findings: Research efforts are continuing to determine if there is a relationship between anti-sphingolipid antibodies which appear in some of these sera and various demyelinating disease states. Data obtained in the course of this study indicated that anti-ganglioside antibodies appear in the serum in approximately 20% of patients with demyelinating diseases such as multiple sclerosis and viral encephalitis. It has been found that various preparations of gangliosides exhibit distinct differences in antigenic potency.

Significance: Since ganglioside preparations vary in antigenic potency, it is important to try to discover the reason for this finding since it may be an important clue to the cause of the difference in levels of anti-ganglioside antibody in these conditions.

Part A continued

Proposed Course: Gangliosides will be prepared and exact analyses performed including experiments to determine if they contain peptides. Traces of the latter material may be quite important as far as the antigenicity of the gangliosides is concerned. When these tests have been completed, the immunology of the rigorously characterized materials will be investigated.

Part B included: No

Serial No. NDB(I)-65 LNC/LC 1241
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Investigation of the Anti-cancer Activity of Derivatives of Tetrolic Acid

Principal Investigator: Dr. R. O. Brady

Other Investigators: None

Cooperating Units: Stewart R. Humphreys, NCI, DE

Man Years: 0

Project Description:

Objectives: To determine if various thioester derivatives of tetrolic acid possess anti-tumor activity.

Methods: Thioester derivates of tetrolic acid will be prepared chemically. The ability of these materials to inhibit growth of Ehrlich's mouse ascites tumor and mouse leukemia L-1210 will be examined.

Major Findings: The project has just been initiated.

Significance: Tetrolyl-coenzyme A has been shown to be a very effective inhibitor of long chain fatty acid synthesis (Robinson, J. D., Brady, R. O., and Bradley, R. M., J. Lipid Res., 4: 144-150 (1963)). The effectiveness of this anti-metabolite as an anti-neoplastic agent will be examined.

Proposed course: To implement this project as outlined.

Part B included: No

Serial No. NDB(I)-61 LNC/LC 816
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Structural and Metabolic Studies of Gangliosides

Principal Investigator: Dr. J. N. Kanfer

Other Investigators: Drs. R. O. Brady and A. Gal

Cooperating Units: None

Man Years: (Computed for the 12 month period)

Total:	1.9
Professional:	0.5
Other:	1.4

Project Description:

Objectives: To investigate the structure and metabolism of gangliosides.

Methods: Structural studies will be performed by chemical syntheses and by enzymatic hydrolyses of gangliosides. Enzymes will be induced in soil bacteria by enrichment culture and extracted from these organisms for degradative studies. Potential precursors of gangliosides will be labeled by chemical syntheses. These materials will be examined in various cerebral enzyme preparations with regard to their role in ganglioside synthesis.

Major Findings: Two potential radioactive sphingolipid precursors of gangliosides have been synthesized in this laboratory: 1, glucocerebroside-^{14}C and glucopsychosine-^{14}C. Metabolic studies with these materials indicate that the former compound is not on the pathway of ganglioside synthesis. These findings indicate that alternative biosynthetic routes and precursors must be examined.

Significance: Studies in this laboratory indicated that the so-called "Tay-Sachs'" ganglioside probably is a normal intermediate in ganglioside formation (Kanfer, J. N., Blacklow, R. S., Warren, L., and Brady, R. O., Biochem. Biophys. Res. Comm., 14: 289-291 (1964)). This finding helps to pinpoint the metabolic abnormality which occurs in Tay-Sachs' disease, i.e., it may be either a

Part A continued

failure to complete the normal ganglioside molecule by the addition of a final molecule of galactose or a defect in ganglioside catabolism. Investigations are now underway to decide between these alternatives

Proposed Course: The catabolism of gangliosides will be investigated using enzyme systems from brain tissue of various sources. Further precursors of gangliosides will be synthesized chemically. In particular, an attempt will be made to prepare ^{14}C-labeled sphingosine by chemical methods. This compound must be the direct precursor of gangliosides. The availability of the labeled material should afford the most direct approach towards elucidating the pathway of ganglioside formation.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B

Publications:

1. Kanfer, J. N. and Young, O.: Observations on cerebroside metabolism *in vivo*. *J. Biol. Chem.* 240: 609-612 (1965)

Serial No. NDB(I)-61 LNC/AAC 810
1. Neurochemistry
2. Proteins and Amino Acids
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Metabolism of free and protein-bound amino acids in neural tissues

Principal Investigator: Dr. D. B. Tower

Other Investigators: Mr. E. L. Peters, Mr. C. J. Lauter

Cooperating Units: Dr. J. F. Hoffman, NHI, LKEM

Man Years (Computed for the 12 month period)

Total:	0.9
Professional:	0.7
Other:	0.2

Project Description:

Objectives: To investigate the metabolic interrelationships (and factors affecting them) of amino acids, especially of the glutamate and aspartate group, in the free and protein-bound pools of neural tissues, with emphasis on cerebral cortex studied in vitro.

Methods: See previous reports and below.

Major Findings: (1) Further studies on proteins isolated from subcellular fractions of cat cerebral cortex and cat liver have been completed. As indicated in the 1963-64 report, attention has been devoted to the microsomal fraction, which has been sub-fractioned into its ribosomal (25%) and membrane (75) components. Previously reported manometric analyses for glutamyl and aspartyl residues have been compared with analyses by the Moore-Stein technique on a Phoenix Amino Acid Analyzer. Agreement for 5 distinctly different samples has been excellent, so that previous data obtained by manometric methods only is amply validated.

Subfractionation of liver microsomes appears to have been superior to analogous studies on brain microsomes. Moore-Stein analyses of brain and liver ribosomal subfractions give amino acid patterns essentially identical to those reported by Crampton & Petermann (J.B.C. 234:2642, 1959). The membrane subfraction, obtained by solubilization with deoxycholate followed by precipi-

Part A continued

tation with trichloracetic acid, shows a distinctly different amino acid composition: acidic residues 19.4% of total N (vs. 11.9 for ribosomes); basic residues 15.4% (vs. 42.5%) and neutral residues 65.3% (vs. 45%).

The deoxycholate (membrane) subfraction was further fractionated on a DEAE-cellulose column. Ten peaks were identified spectrophotometrically. Based on previous observations by Dr. Trams, peaks 7, 8 and 9 were chosen as most likely to show significant excess of acidic residues. Peaks 7 and 8 differed little, in this respect, from the unchromatographed membrane subfraction, but peak 9 was distinctly different. This peak, calculated to represent about 20% of the total material chromatographed, shows 30% acidic residues, 5% basic residues and 65% neutral residues. Thus, approximately 1/3 of the total amino acid residues are here contributed by glutamyl and aspartyl residues, and the protein(s) would be expected to exhibit a marked excess of anionic charges. In this respect it closely resembles the eel electroplax microsomal subfraction P-12 reported in 1963-64. If this peak-9 material represents a single protein, the minimal, calculated molecular weight would be about 153,000 with 1220 amino acid residues. The results of these studies strongly support our earlier suggestion that the membranes of the endoplasmic reticulum contain proteins with a substantial excess of negative (anionic) charges, a characteristic consonant with roles in transmembranal transport.

(2) In collaboration with Dr. J. F. Hoffman (NHI:LKEM) similar amino acid analyses have been carried out on sheep erythrocyte ghost proteins. The erythrocytes were obtained from genetically pure strains of sheep exhibiting either low K^+ or high K^+ erythrocytes. Preliminary results indicate a distinct difference in the amino acid composition of the two types of erythrocyte membrane proteins. Proteins from the high K^+ cells show significantly less seryl and more leucyl residues than the proteins from low K^+ cells. Confirmatory studies with additional ghost samples are now underway. A comparison of the sheep erythrocyte data with those previously obtained on human erythrocytes (see 1963-64 report) indicate that the glutamyl and aspartyl residues in the proteins from both species of cells are of similar orders of magnitude.

(3) Investigations by Mr. Peters on the *in vitro* metabolism of methionine by cerebral cortex slices from normal and methionine-sulfoximine-intoxicated cats are continuing. Previous results obtained by paper chromatography have been verified quantitatively by Moore-Stein analysis of slice, free-pool constituents. These analysis have indicated some difficulties with the transamination reactions for α-keto acid assay, so that an improved procedure utilizing *E. Coli* transaminases is being adapted. Data on specific

Part A continued

activities of intermediates in the pathway of methionine to glutamate will shortly be available with our newly acquired liquid scintillation flow cell in conjunction with the amino acid analyzer. Progress in this respect has been slowed by the necessity of adapting and standardizing the techniques for these new analytical tools

Significance: This project bears directly upon the fundamental problem of delineating some of the interrelationships between cerebral structure (proteins) and metabolism, and between metabolism and functional activity, so that an eventual integration of these facets of cellular neurochemistry can be realized.

Proposed Course: To continue these studies along lines indicated by present results.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B

Publications:

1. Tower, D. B.: Tenkan no Seikagaku (Biochemistry of Epilepsy) translated from the 1960 English edition into Japanese by A. Mori, M. Takasaka and S. Nishimoto) Osaka, Nagai-Shoten, 1964, 306 pp.

2. Tower, D. B.: Arrest of seizure activity: biochemical aspects and pharmacology. Epilepsia, in press.

Serial No. NDB(I)-61 LNC/AAC 811
1. Neurochemistry
2. Protein and Amino Acids
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Electrolytes and energy metabolism in cerebral cortex in vitro

Principal Investigator: Dr. D. B. Tower

Other Investigators: Mr. E. L. Peters

Cooperating Units: Drs. J. F. Hoffman and M. Burg, NHI, LKEM

Man Years (Computed for the 12 month period)

Total:	1.2
Professional:	1.1
Other:	0.1

Project Description:

Objectives: To study the in vitro metabolism of electrolytes and of energy-producing cyles and compounds thereof in incubated slices of cerebral cortex.

Methods: see previous reports and below.

Major Findings: (1) On the basis of data reported in the 1963-64 report, a limited study of K^+ exchange and turnover in incubated slices of cat cerebral cortex was undertaken. It was recognized that the factor of "extracellular" space was not yet definable with certainty, but a direct confrontation of the problems involved with use of radioion tracers seemed worthwhile at this stage.

Studies were carried out essentially as previously reported by Krebs et al (1951) and Cummins and McIlwain (1961), i.e. preincubation of slices (here in 27 mM K^+ media) to stable levels of slice swelling and K^+ maintenance (at 30 min. incubation, aerobically, 37°) followed by addition of 0.1 μc. K^{42} and continued incubation for 10 to 25 min. longer, followed by appropriate analyses of slices and of media. Parallel studies were carried out with slices incubated in media containing 10^{-5} M ouabain, where cation transport had been shown to be completely inhibited.

On analysis it was found that K^{42} uptake by ouabain-inhibited slices reached plateau and maximum K^{42} specific activity within

Part A continued

less than 10 min. after K^{42} addition. Comparison of 10 vs 20 min. slices under these conditions indicated no transport of K^{42} into slices between 10 and 20 min, as expected if ouabain inhibition were complete. In contrast normal slices exhibited a steady uptake of K^{42} with maximum slice specific activity attained at 25-30 min. after K^{42} addition and definite evidence of K^{42} transport into slices. Uncorrected calculations of K^+ exchange and turnover by the method of Krebs et al (1951) did not give similar rates for the 10 vs. 20 min. observation periods and were thus inconsistent with observed steady-state maintenance of slice K^+. Hence it was obvious that a multicompartment system must be involved (as originally anticipated); when the above rates were recalculated on the assumption of a two-compartment model, consisting of a rapidly equilibrating "extracellular" space and a more slowly equilibrating intracellular compartment, constant exchange and turnover rates for the latter were obtained. The respective rates averaged 0.64 μeq./min. and 6.25% of total IC K^+/min., values in good agreement with those of Cummins and McIlwain (1961) obtained on "stimulated" slices (theirs by electrical pulses, ours by extra K^+ in media).

Two points of interest emerged from these studies: (a) slice K^{42} specific activities failed to reach 100% of the medium S.A. (slice average about 85%), suggesting the presence in slices of a slowly exchangeable K^+ fraction, as previously suggested by Katzman (1953) from in vivo studies; and (b) ouabain-inhibited slices behaved toward added K^{42} as one, rapidly exchangeable K^+ pool (except for the fraction cited in (a)) and hence apparently freely permeable to monovalent cations.

(2) The effects of 10^{-5}M ouabain on respiration and glycolysis of incubated slices of cat cerebral cortex were investigated. The significance of this study derives from the previous observations: (a) of Whittam (1961 et seq.) that ouabain-inhibited tissues from cerebral cortex, liver, kidney and erythrocytes exhibit inhibition of Na^+ and K^+ transport, inhibition of Na-K-ATPase, and parallel inhibition of oxygen consumption (or glycolysis) - hence cation transport and the ATPase subserving it regulate a significant fraction (30-50%) of tissue, suprabasal oxidative metabolism; and (b) of Schwartz (1962) who failed to confirm Whittam's observations on oxygen consumption, but found, instead of inhibition, a stimulation in the presence of ouabain, and suggested that the discrepancy might relate to the presence (Schwartz) or absence (Whittam) of Ca^{++} in the incubation medium.

Our studies completely confirmed Schwartz's suggestion. In bicarbonate-buffered media plus Ca^{++}, the presence of 10^{-5}M ouabain stimulated oxygen consumption 40% but depressed aerobic glycolysis 54%; whereas in the same medium minus Ca^{++}, the effect of ouabain was depression of oxygen consumption 20% and of glycolysis 37%.

Part A continued

The latter findings were confirmed in a Ca^{++}-free, phosphate medium, and the manometric data were verified by direct analyses of glucose consumption and lactate production.

One of two conclusions seem indicated: either Whittam's hypothesis is wrong (i.e. cation transport does not exert a regulatory effect on oxidative metabolism) or there are effects of ouabain in addition to those on Na^+ and K^+ transport. Since Whittam also showed effects on transport, ATPase and oxygen uptake in Na^+-free media, the second conclusion seems more likely. In fact, in cardiac muscle Holland (1963, 1964) has reported that ouabain mobilizes tissue-bound Ca^{++}, and a number of studies by Chance (1963), Brierly (1963, 1964) and Lehninger (1964) indicate that excess Ca^{++} stimulates mitochondrial oxygen uptake by a mechanism rather analogous to uncoupling of oxidative phosphorylation. These latter observations would seem to offer a reasonable explanation for the ouabain effects on cortical respiratory metabolism which we have observed.

(3) In order to understand the meaning and significance of the data on swelling and fluid spaces in cerebral cortex slices reported in the 1963-64 report, it seemed essential to extend those studies to cerebral tissue preparations with distinctly different morphology, namely, sub-cortical white matter (glial cells and myelinated axons only) and monatal cerebral cortex (deficient in adult complement of glia, especially astrocytes). The latter were chosen first because of greater technical ease of handling.

Repetition of the studies previously done on adult cat cerebral cortex was carried out on neonatal (60-90 hr. after birth) kitten cerebral cortex. Striking differences have been observed: neonatal slices show no swelling (vs. adult swelling of 32%); neonatal slices show only one sucrose space (vs. two in adult slices) which is only slightly smaller than the chloride space (45% vs. 52%); and neonatal slice Na^+ and K^+ contents are distinctly different than the adult. The significance of these observations must await electron micrographs of the kitten cortex, but it is suspected that a major factor is the relative lack of astrocytes in neonatal kitten cortex (as suggested by Brizee and Jacobs (1959)).

(4) Attempts to extend studies previously reported on cat cerebral cortex slices to slices of human cortex samples obtained at neurosurgical operation met with a number of difficulties. The human samples appeared to be already swollen with smaller than predicted chloride and sucrose spaces. Inquiry revealed that excision had been lengthy with prolonged ischemia of the biopsy samples.

A controlled study was then undertaken to assess the effects

of circulatory arrest. Cats were dispatched in the usual manner and the removal of the brain for slicing and incubated delayed 30 min. thereafter. Incubated cortical slices from these brains failed to swell (10.5% vs. the usual 33%) and exhibited smaller chloride spaces (56% vs. 64%) and decreased slice K^+ (81 μeq./g. vs. 100 μeq./g.). Biopsy samples of such brains showed a significant decrease in solids (13.5% vs. 16%), consistent with about 18.5% swelling in situ, as well as a normal Cl^- space, increased CSF Cl^- (137 vs. 130) and K^+ (29.5 vs. 3.8) and decreased tissue K^+ (81 vs. 94.5) and increased tissue Na^+ (66 vs. 57). These results are consistent with those suggested by Van Harreveld and are quite analogous to those observed during spreading cortical depression by Van Harreveld, Marhsall, Bures and others.

Significance: Energy-yielding metabolism is the basic factor underlying neuronal function and activity, and electrolyte metabolism (which clearly depends upon it) provides a fundamental link between cellular chemistry and the functional activity of impulse conduction. The understanding of the factors involved is essential for the elucidation of both normal functioning of neural tissues as well as deranged function of hyperactivity states associated with concussion and edema.

Proposed Course: To pursue investigations along lines indicated from current major findings.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B

Publications:

1. Bourke, R. S., Greenberg, E. S. and Tower, D. B.: Variation of cerebral cortex fluid spaces in vivo as a function of species brain size. Am. J. Physiol., 208: 682-692 (1965).

2. Tower, D. B.: Problems associated with studies of electrolyte metabolism in normal and epileptogenic cerebral cortex. Epilepsia, in press.

3. Bourke, R. S. and Tower, D. B.: Effects of ouabain on electrolyte and respiratory metabolism in slices of cat cerebral cortex incubated in vitro. Biochem. J., in press.

Serial No. NDB(I)-62 LNC/PM 941
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Studies on Bioelectrogenesis

Principal Investigator: Dr. E. G. Trams

Other investigators: Mr. C. J. Lauter, Mr. C. Hoiberg

Cooperating Units: Drs. R. O. Rudin and P. Mueller, Eastern Pennsylvania Psychiatric Institute, Philadelphia

Man Years (computed for the 12 month period)

Total:	1.2
Professional:	1.0
Other:	0.2

Project Description:

Objectives: This is a combination of related studies dealing with the mechansisms involved in the generation and propagation of electrical impulses in the cell. A collaborative study with investigators at the Eastern Pennsylvania Psychiatric Institute is concerned with the synthetic approach, namely, reconstitution of excitable cell membranes or larger cell units from identified chemical components. The present objectives are (1) the identification of the structures and mechanisms involved in bioelectrogenesis and (2) reconstitution of the components to form functional units which exhibit part or all of the functions observed *in situ*.

Methods: See 1962 Report.

Major Findings: The analyses of the constituents of electric tissue have been continued. The characteristics of a number of low-molecular protein fractions obtained from the electric organ of *Electrophorus electricus* have been established. Special attention was paid to a material which was identified as a complex sialo-glycoprotein. This glycoprotein showed a number of features which are considered essential in receptor site chemistry. The interactions of this protein with various biogenic amines and

Part A continued

and quaternary compounds was investigated. The results obtained indicated that, although this material served reasonably well as a model for receptor proteins, it could not be regarded as one of the actual receptors (such as the one postulated for acetylcholine) which appear to be of significance in the bioelectrogenic process.

Further attempts to fractionate the endoplasmic reticular components with high binding activity for quaternary ions have not been successful. On the basis of most of these experiments it has been concluded that receptor function must be "sitic" in nature, namely that the geometry of the site confers specificity rather than its constituent molecules alone.

In order to gain a better insight in the structure and function of the postulated bi-phasic (both lipophilic and hydrophilic components) receptor site, the composition of the lipids of the electric organ of electrophorus was investigated. The lipids from the electric tissue, some of its subcellular fractions and, for comparison, from striated muscle of the eel, were extracted. The total lipid fractions were then subjected to silicic acid column chromatography and the fractions were isolated and analyzed for their lipid composition.

The various lipid fractions thus obtained were tested in the Rudin-Mueller system for their capacity to form bimolecular lipid leaflets in an aqueous phase. It was found that the unfractionated lipids of the electric organ and the fractions which contained a mixture of polar and non-polar lipids served best to form bilayers with maximum stability. The capacity and dielectric breakdown strength of the bilayers formed were estimated. Interaction with the EIM (Excitability Inducing Molecule) of Mueller and Rudin was tested. Although in most cases membrane resistance was lowered following the addition of the EIM, no excitable membranes could be formed with the lipid fractions obtained by this method.

Significance: These studies are a part of a collaborative attempt to reconstruct an excitable membrane with some of the characteristics of a true plasma membrane. Data obtained by analytical methods are used in a stochastic method to arrive at probable membrane compositions, i.e. membranes which are sufficiently stable, allow for some ionic discrimination, etc. The purely analytical studies on some of the active tissue fractions will aid in the understanding of the composition of electric tissue, and its differences from other tissues, differences which might contribute to the elucidation of the bioelectrogenic process.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B:

Publications:

1. Trams, E. G.: Properties of Electroplax Protein II. Biochim. Biophys. Acta 79: 521-530 (1964)

2. Trams, E. G. and Lauter, C. J.: Properties of electroplax protein III. Biochim. Biophys. Acta 83: 296-304 (1964)

3. Brady, R. O. and Trams, E. G.: The chemistry of lipids. Ann. Rev. Biochem., 33: 75-100 (1964)

Serial No. NDB(I)-65 LNC/PM 1242
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The mechanism of lipoprotein synthesis

Principal Investigator: Dr. E. G. Trams

Other Investigators: Mr. C. J. Lauter

Cooperating Units: Dr. E. A. Brown, Lab. Chem. Pharmac., NHI

Man Years: (Computed for the 12 month period)

Total:	1.1
Professional:	1.0
Other:	0.1

Project Description:

Objectives: To investigate mechanisms of plasma lipoprotein synthesis as potential models for membrane construction and organization.

Methods: Lipoprotein synthesis is studied using established methods. The various lipoprotein fractions are isolated by flotation in the ultracentrifuge, or are separated by electrophoresis. Formation of the lipoproteins has been studied in dogs and in rats. Isotopic precursors are administered to the animal to label either the protein moiety or the lipid moiety of the lipoprotein molecule. In some instances, the protein portions of separated lipoproteins were labeled with fluorescent dye and the fluorescent lipoproteins were reinjected into the animal. Carbon tetrachloride has been used to specifically inhibit lipoprotein synthesis in the liver. For a number of experiments the synthesis of lipoproteins was studied in vitro with liver slices or cell-free preparations from liver homogenates.

Major Findings: Attempts were made to recover plasma lipoproteins from the liver after administration of isotopic precursors. So far, we have been unable to obtain lipoproteins with the characteristics of those circulating in the plasma. It was found, however, that the liver will synthesize lipo-protein-like materials from C^{14}-methionine or from leucine-C^{14} or palmitic acid C^{14}. Certain phases of lipoprotein synthesis were not

Part A continued

affected by carbon tetrachloride. The possibility that CCl_4 acted as a competitive inhibitor for lipid carrying sites in plasma lipoproteins was excluded.

Further studies on the incorporation of C^{14}-methionine indicated the possible formation of a low molecular weight, lipid-soluble peptide as a precursor. It has been postulated that such a peptide (or peptides) act as lipid-specific carriers which determine the lipid composition of the lipoprotein molecule to be formed.

Isotopic studies and experiments with fluoresceine labeled lipoproteins indicated that the plasma half-life of the lipid portion and the protein portion of the lipoprotein molecule was not identical. It was observed that the half-life of the low density lipoproteins was very short, while that of the high density class was relatively long, namely several days. Attempts were made to demonstrate a possible interconversion of one lipoprotein class into another; the results obtained so far indicate such a mechanism to be probable but further data are required to substantiate this conversion. Preliminary analyses of the amino acid composition show that the peptide portion of the lipoproteins can be divided into two different families. The most abundant amino acids in all lipoproteins isolated from rat plasma were leucine and lysine.

Significance: The investigations outlined above not only will yield a better insight into the structure and the metabolism of the plasma lipoproteins but should also indicate by what mechanisms peptides and lipids aggregate into definite complexes of biological significance. This project has evolved from some experimental observations and through some of our current thinking on the structure and function of the plasma membrane. We have postulated that the membrane is composed of a lipid-bilayer with protein covering certain portions of the bilayer and also interdigitating with the lipid phase in some areas. The concept was developed, that a template mechanism or a coding principle exists which will determine the composition of the lipid phase. It was reasoned that a similar, if not identical, problem exists in the formation of the plasma lipoproteins. The study of lipoprotein synthesis appeared a logical approach to this problem and was selected since, a facile experimental approach seemed feasible.

Proposed Course: It is proposed to continue this investigation along the lines described above. Further studies will be made of the *in vivo* and *in vitro* incorporation of isotopic precursors. It is proposed to fractionate some of the lipoproteins after their isolation into their lipid and peptide portions and to investigate the metabolic fate of the latter. Since the methodology for the

Part A continued

study of lipoproteins leaves much to be desired it is proposed spend considerable effort in improving the available methods and to search for new approaches for lipoprotein isolation or separation.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B

Publications:

1. Brown, E. A., Lauter, C. J. and Trams, E. G.: Studies on the mode of action of carbon tetrachloride. Life Sciences (in press)

Serial No. NDB(I)-65 LNC/PM 1243
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Pilot studies and consulting services in neurochemistry

Principal Investigator: Dr. E. G. Trams

Other Investigators: Mr. C. J. Lauter

Cooperating Units: (see below)

Man Years (computed for the 12 month period)

Total:	0.6
Professional:	0.5
Other:	0.1

Project Description:

Objectives: To provide for various NINDB-IR programs and investigators a focal point for consultation, advice and assistance on neurochemical and biochemical aspects of research problems. To develop new approaches and test new methods; to study non-recurring problems amenable to current biochemical approaches; and as appropriate, to train and/or supervise technical or sub-professional personnel and research associated in such areas.

Methods: As required (see below).

Projects Prosecuted during FY 65:

1. For SN-Section on Child Neurology (Dr. Dekaban): Analyses of patient's urine were performed for the presence and identity of mucopolysaccharides. Patient material was predominantly from children with Gargoylism. A method for isolation and identification of urinary mucopolysaccharides was selected, tested and recommended to the above investigator. Some preliminary studies on thin-layer chromatography of mucopolysaccharides were carried out. Standards were chromatographed in a number of solvent systems on a variety of support media, but results so far are not satisfactory.

2. For SN-Neurosurgery (Dr. Li): Advice was provided on design details which would facilitate blood gas studies on animals in a proposed hyperbaric chamber.

3. For SN-Section on Primate Neurology (Dr. Ommaya): Instruction in techniques for analyses of cerebral samples for electrolytes (Na^+, K^+, Cl^-) were given for future use in a project on experimental concussion in monkeys.

4. For LNP-Section on Spinal Cord (Dr. Nelson): Hemolymph and tissue extracts of Cepaea nemoralis were analyzed for major electrolytes and solutes by standard microanalytical techniques. A buffered, artificial hemolymph for use with isolated ganglion cells during neurophysiological recordings was designed and recommended to the above investigator.

5. For OB-Section on Ophthalmology Pharmacology (Dr. Macri): Protein analyses by the Lowry micro-method were carried out on a set of aqueous humor samples and results reported to the above investigator.

6. For OB-Section on Cell Biology (Dr. Bonting): A technician from this section was instructed in the use of the Cotlove-Bowman chloride titrator and an instrument in LNC was made available for her to run chloride analyses on samples required by the above investigator.

7. For MN (Dr. Jacob): Advice on various methods for histochemical analyses of spinal cord sections from ALS patients for lipochrome was provided.

8. For MN-Section on Applied Pharmacology (Dr. Irwin): Advice on substitutes for chloride ions in muscle perfusates was provided. The isethionate ion was recommended and a sample for testing was provided.

9. For NIMH-AP-Section on Psychosomatic Medicine (Dr. Coburn): Instruction in use of the LNC atomic absorption flame spectrophotometer and availability of the instrument were provided for trace metal analyses in brain tissue from experimental animals. This made possible an extensive series of analyses for Ca^{++}, Mg^{++}, Cu^{++}, Zn^{++}, Fe^{+++}, etc.

10. For NHI-LM (Dr. Fredrickson): Current concepts of the occurrence, chemistry and physiological significance of neural glycolipids was reviewed.

11. For NHI-LB (Dr. Hendler): A preliminary investigation of the occurrence and structures of lipoamino acids was begun. Materials were isolated from hen oviduct following incubation with labelled amino acids, and non-polar fractions were separated by silicic acid column chromatography. Resolution of the lipoamino acid mixtures was checked by thin-layer chromatography in various solvent systems. Methods to test for various functional groups in these compounds are under development.

12. For NHI-LKEM (Dr. Hoffman); A limited number of protein hydrolysates from genetically specific, sheep erythrocytes (ghosts) were analyzed for amino acid composition on the LNC amino acid analyzer.

Significance: The types of activities exemplified above represent

an attempt by the Laboratory of Neurochemistry to provide for training in techniques and analytical requirements (on a limited scale) as well as advice and consultation for those laboratories and branches lacking appropriate personnel, instrumentation and the like for important biochemical aspects of their own projects. Such activities are in addition to and complement the usual collaborative projects between such groups and LNC investigators.

Proposed Course: Continuation as outlined above under "objectives".

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B

Publications:

1. Fredrickson, D. S. and Trams, E. G.: Ganglioside lipidoses (Tay Sach's disease; infantile amaurotic familial idiocy). In Stanbury, J. B., Weingarden, J. B. and Fredrickson, D. S. (Eds.): Metabolic Bases of Inherited Diseases (ed. 2). New York, McGraw-Hill, 1965 (in press).

2. Trams, E. G., Lauter, C. J., Bourke, R. S. and Tower, D. B.: Composition of Cepaea nemoralis hemolymph and tissue extracts. Comp. Biochem. Physiol., 14: 399-404 (1965).

Serial No. NDB(I)-61 LNC/AAC 812
1. Neurochemistry
2. Proteins and Amino Acids
3. Bethesda, Md.
Terminal Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Isolation and Characterization of chicken brain, liver, and erythrocyte chromatins.

Principal Investigators: Drs. C. W. Dingman and M. B. Sporn.

Note: Project terminated as a result of transfer of both principal investigators to NCI on 7/1/64.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B

Publication:

1. Dingman, C. W. and Sporn, M. B.: Studies on chromatin I. Isolation anc characterization of nuclear complex of deoxyribonucleic acid, ribonucleic acid, and protein from embryonic and adult tissue of the chicken. J. Biol. Chem. 239:3483-3492 (1964).

Serial No. NDB(I)-61 LNC/PM 814
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.
Terminal Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Studies on enzyme synthesis

Principal Investigator: Dr. E. G. Trams

Other Investigators: Mr. C. J. Lauter

Cooperating Units: Dr. D. Fredrikson, ODR, NHI

Man Years (Computed for the 12 month period)

Total:	0.1
Professional:	0.1
Other:	0

Project Description:

Objectives: To investigate factors involved in the synthesis of soluble and particulate enzymes.

Methods: Standard procedures.

Patient Material: Obtained on patients admitted for other purposes.

Major Findings: See 1963 report. Only 1 additional patient has been available. Results obtained were comparable to 5 previous cases.

Significance: These studies have been designed to further our understanding of the mechanisms which are involved in the synthesis and control of enzymes. The relevance of such studies to enzymatic aspects of disordered metabolism is obvious.

Proposed Course: Termination, because of insufficient clinical material.

Part B included: No

Serial No. NDB(I)-61 LNC/LC 818
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md
Terminal Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Redox Phenomena in Nerve Conduction

Principal Investigator: Dr. R. O. Brady

Other Investigators: Dr. I. Tasaki, LNB, NIMH

Cooperating Units: LNB, NIMH

Man Years (Computed for the 12 month period)

Total: 0

Project Description:

Objectives: To investigate the biochemical mechanisms underlying nerve conduction, and in particular to determine whether or not conduction of impulses by nerve tissue is associated with a biological oxidation-reduction process.

Methods: Micropipettes were inserted intra-axonally into giant squid axons through which specific sulfhydryl group inhibitors were introduced during standard recordings of axonal conduction.

Major Findings: Various sulfhydryl inhibitors such as N-ethyl-maleimide and iodoacetamide had no effect on the instantaneous processes involved in impulse conduction. The finding is in keeping with similar results obtained earlier in experiments using p-chloromercuribenzoate, another sulfhydryl reagent.

Significance: It appears from these experiments that redox phenomena involving the formation and rupture of disulfide bonds is not intimately concerned with impulse conduction in nerves. These findings are of considerable interest in view of the marked arteriovenous difference in reduced glutathione level of cerebral blood. Furthermore, the data seems to exclude a configurational change in the nerve membrane, during impulse propagations as far as disulfide bonds are concerned.

Proposed Course: Termination of the project.

Part B included: No

ANNUAL REPORT
July 1, 1964 through June 30, 1965
Laboratory of Molecular Biology
National Institute of Neurological Diseases and Blindness
Intramural Research

Ernst Freese, Chief

The research of the laboratory can be roughly divided into two parts, although the overlap between the two has made the studies especially fruitful. A. Structure and Alteration of Nucleic Acids, which includes studies on the evolution of genes. B. Control Mechanisms and Differentiation. Enzymic control mechanisms are the fundament of differentiation, whereas sporulation and germination represent an especially simple example of differentiation which can be studied by a combination of biochemical and genetic methods.

A. Structure and Alteration of Nucleic Acids

1. Mutagenic and Inactivating Effects of Hydroxylamine and Its Derivatives.

In earlier studies, hydroxylamine had been found to be highly mutagenic with little concomitant inactivating effect when it was used at high concentrations (1 M). At low concentrations, however, it exerted a strong inactivating effect without inducing mutations. Initially, these studies involved phages for which a lethal effect can always be attributed to the alterations of the protein coat. However, when the same lethal effect was observed also for transforming DNA, it became clear that a direct action on DNA was involved. A thorough investigation has now established the following facts. Whereas the mutagenic effect is directly caused by the reaction of hydroxylamine with the cytosine bases of DNA, the inactivating effect comes about indirectly; hydroxylamine, or its derivatives that have a free NOH group, reacts with oxygen (by means of a metal catalyst) to form a compound which can attack both thymine and guanine in DNA and breaks these bases open. DNA can subsequently not duplicate across the opened base, an effect which is lethal for transformation and may induce large chromosomal alterations in higher organisms. These findings are especially significant since all NOH containing compounds, which have a lipophilic group (enabling them to enter cells), are highly carcinogenic. It is therefore very likely that chromosomal breaks induce cancer. The techniques developed in this investigation make it now possible to rapidly screen many other compounds for their direct and indirect chemical and lethal effect on DNA.

It is intended to determine whether the reaction product of hydroxylamine can directly break the sugar phosphate backbone of DNA or whether the observed breakage occurs indirectly, either chemically or enzymically, as a consequence

of the alteration of DNA bases. In order to enlarge the scope of this investigation other chemicals which are also mutagenic, lethal, and carcinogenic will be examined by the above methods.

2. Studies on the Structure of Chromosomes.

The DNA strands which one isolates from cells must be attached lengthwise to one another in chromosomes, because genetic markers are one-dimensionally arranged. It is not known, however, whether or not there are any non-DNA units that link DNA molecules. Such links have been postulated for various reasons which are not decisive, and they have been suggested by experimental results which are not convincing. One argument, for example, has employed the observation that hydroxylamine causes DNA breaks. The authors had not observed any reaction with DNA itself and concluded that some peptide links must be broken. The above finding of a reaction of hydroxylamine with the DNA bases renders this argument invalid.

The decision of whether or not there exist links between DNA is of great importance to the understanding of mutations, chromosome duplication, and especially chromosome function. For it is not known which special properties of a chromosome designate the regions of DNA that should be used for the synthesis of messenger RNA. The problem of DNA links will be mainly studied by a guest worker who has just joined the laboratory.

3. Genetic Analysis of Virus-Induced Mutants of Escherichia coli.

The properties of virus μl, which lysogenizes E. coli bacteria and simultaneously mutates them, have been further investigated. The lysogenic state is normally stable because the viral DNA is inserted into the bacterial chromosome. This insertion has been proven by the fact that the genetic distance of markers right and left of the phage attachment site is larger for the lysogenic than for the non-lysogenic state. Wherever the phage is inserted, the corresponding enzyme is absent and the function of the operon interrupted, as has been shown for the β-galactosidase operon which comprises three enzymatic properties. When the virus DNA is incorporated into the β-galactosidase gene the activity of the genes distal to the operator site is also eliminated, apparently because the corresponding messenger RNA can no longer be made. However, when the virus DNA is incorporated into one of the other two genes of the β-galactosidase operon, the function of β-galactosidase is normal and shows the normal induction properties. Recombinants from crosses between lysogenic and non-lysogenic strains often produce strains whose lysogenic state is unstable; in these strains the phage DNA apparently persists as an extrachromosomal element without initiating virus multiplication.

4. The Role of Mutation in Evolution.

Mutations can be subdivided into different classes according to the mechanisms by which they arise. Since large alterations usually result in rather drastic (often lethal) changes, most mutations that persisted during evolution must have involved the change of a single nucleotide pair of DNA. One can show that mutations of the transition type, i.e., changes $\frac{G}{C} \longleftrightarrow \frac{A}{T}$

must have predominated, at least in vital proteins. The knowledge of the most frequent chemical changes of DNA suggests further that the changes from G → A should have occurred much more frequently than those in the opposite direction. Since the code between nucleic acids and proteins is now being unraveled it will soon be possible to check from the known sequence of amino acids in protein whether the above picture is correct and whether the same types of mutations have predominated in all organisms.

During the evolution of bacteria at least half of the DNA bases must have mutated once. Throughout this process enzymic properties may have changed and new enzymes may have been formed. In particular, enzymes like dehydrogenases which have a common cofactor, NAD, may have evolved from a common precursor. The examination of three dehydrogenases from Bacillus subtilis has indeed shown that they are functionally, immunologically and structurally related to one another.

The enzymes investigated are alanine (AlD), lactic (LDH), and malic (MDH) dehydrogenase. Although their molecular weights differ, their subunits all have a molecular weight of about 35 000, AlD and MDH crossreact immunologically with each other and, although weaker, with LDH. AlD and MDH have at least four peptides in common, two of which occur also in LDH.

B. Control Mechanisms and Differentiation

1. Initial Stages of Germination.

Germination of Bacillus spores is specifically initiated by L-alanine. The spores release dipicolinic acid and calcium, which have formed a protecting coat, and subsequently start the synthesis of polymer molecules. In order to elucidate the mechanism by which alanine initiates germination, mutants of B. subtilis have been isolated which were deficient in alanine dehydrogenase. AlD was the only known enzyme that could be involved in germination, since it would convert L-alanine into pyruvic acid and thereby possibly initiate energy production via the Krebs cycle. It was found that AlD deficient mutants germinated at a lower rate but they still responded to alanine. Hence, AlD does support germination, but it is not the only enzyme necessary for this process.

Meanwhile new mutants have been found which do not germinate at all in the presence of L-alanine and germinate slowly (12 hours) in a rich medium. Some of these mutants have an intact AlD but at least some of them miss another enzyme that converts α-aminobutyrate to α-ketobutyrate. Since these mutants can still grow in a glucose-minimal medium, the mutation apparently involves a developmental gene. Further genetic and biochemical studies on the normal and mutant enzyme promise to identify the first step of germination.

It may be worth pointing out that an understanding of germination is not only important as a model case of differentiation, but it is also of interest for two other reasons. First, a detailed knowledge of this process is of importance, for example, for the food industry which has to eliminate

Clostridium spores in cans. Second, the process of germination is quite similar to that of fertilization of eggs, which can also be initiated by certain agents instead of sperms (parthenogenesis).

Since various mutations concerned with germination seem to be located in the genetic region of AlD, one may hope to learn, by the same or similar genetic studies, more about the process of AlD induction which was discussed in the previous annual report.

Germination by L-alanine occurs much more rapidly when spores are heated to 70° for 30 minutes before the addition of alanine. This heat treatment can be replaced by exposure of the spores to a saturated solution of calcium-dipicolinate. The Ca-dipicolinate inside can apparently attach to crystals outside the spore and thus liberate certain sites on the spore wall for the action of L-alanine.

2. Control in Synchronized Bacteria.

Bacteria, whose division is synchronized to occur at given times in a mass culture, allow one to examine the development of enzymic processes throughout the mitotic cycle. To accomplish this synchronization a new method has been developed in which B/r bacteria are adsorbed to membrane filters and newly formed buds are continuously eluted. All eluted bacteria are at the same stage of their mitotic cycle and develop synchronously. In these B/r bacteria DNA, RNA, and induced enzyme (β-galactosidase) synthesis proceeded continuously throughout the cycle. Enzyme induction was possible at any time in the cycle and could be stopped immediately by chloramphenicol. Although these results prove that the mitotic cycle does not influence the inducibility of enzymes, they do not seem to be related to the duplication state of chromosomes. For it is likely that the ring chromosomes of B/r bacteria begin their duplication randomly at any position so that all states of chromosomal duplication are present even in a mitotically synchronized mass culture. Genuine chromosomal synchrony has been obtained by other workers, in different bacteria and in yeast, giving rise to synchronous enzyme synthesis. The induction process of enzymes, however, has not been studied in these cases. Such an analysis should now be possible in germinating bacteria which show excellent synchrony.

3. Some Properties of Bacterial Cell Walls.

During the course of studies on control mechanisms certain observations were made which seemed to be worth following up because they revealed some new properties of bacterial cell walls.

Several enzymes, such as RNAase, protease, and amylase, are known to be released into the medium by B. subtilis, whereas in E. coli they could be liberated only by lysozyme or, as recently found, by the exposure to the chelating agent EDTA. Most of the other enzymes naturally remained inside the cell under any conditions in both bacteria. It appeared likely that the different behavior of the two bacteria was caused by a difference in the cell wall. In both bacteria the enzyme would move outside the cell membrane, but in E. coli the cell wall would be thick enough to retain them in the

"periplasm" between cell membrane and wall. One additional enzyme that was found in the periplasm of E. coli was alkaline phosphatase. If the general picture was correct the same enzyme should be released (by an exoenzyme) in B. subtilis. This was indeed observed. As soon as alkaline phosphatase was formed, after derepression in limiting phosphate, it appeared free in the medium.

When a bacterial culture is aerated, until it reaches the resting phase, the bacteria usually become very resistant to lysis by lysozyme. It has been found that certain bacteria, such as E. coli, Clostridia, and B. subtilis, lyse immediately when they are exposed, after lysozyme treatment, to high concentrations of magnesium or other cations. Some components of the cell wall are presumably held together by divalent cations and these bonds can be dissolved by an excess of added cations.

C. Goals for the Coming Year

The structure and alteration of chromosomes will be studied in detail. In particular, it will be attempted to obtain definite evidence for or against the existence of links between DNA strands in higher chromosomes. Inactivating DNA alterations, induced by different chemicals, will be analyzed with respect to their types and their induction of pointmutations and large chromosomal alterations. Furthermore, it will be determined which of these chemicals attack DNA directly and which indirectly.

Mechanisms of RNA synthesis and enzyme induction and repression will be studied both in vivo and in vitro. Enzymic changes and other molecular events that occur in sporulation and germination will be analyzed.

Serial No. NDB(I)-62 LMB/OC 947
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Structure and Alteration of DNA and Chromosomes.

Principal Investigators: Elisabeth Bautz Freese, Ph.D., Ernst Freese, Ph.D., and Richard Welsh, Ph.D. (Guest Worker)

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.5
Professional:	1.5
Other:	1.0

Project Description:

Objectives: 1. Hydroxylamine and its derivatives having a free NOH group exert a strong lethal effect on transforming DNA, whose rate does not change with the hydroxylamine concentration over several powers of 10. In contrast, the mutagenic effect increases linearly with the hydroxylamine concentration. We have elucidated the molecular mechanism of these effects. 2. It is not known whether the DNA pieces in a chromosome are longitudinally held together by non-DNA links or whether DNA exists as one continuous double strand of which one chain is occasionally interrupted. We shall attempt to decide between these alternations.

Methods Employed: For transformation and mutation the same methods were used as in the previous report. For DNA melting and long term alteration of bases a Gilford recording spectrophotometer was employed.

For chromosomal studies: calf thymus nuclei were prepared from frozen calf thymus tissue by washing at subzero temperatures. The DNA fractions were prepared by standard procedures. A polymerizing fraction was obtained from water extracts of nuclei by precipitation with $BaAc_2$. Subunit fractions of DNA from E. coli or S. marinorubra were obtained by first spheroplasting the bacteria with lysozyme, then dialyzing at 0^{o} against a buffer

containing an anion exchange resin. Physical characterizations were made by ultracentrifugation, viscosimetry, and light scattering.

Major Findings: 1. Hydroxylamine and derivatives. a) At high concentration ($\approx$ 1 M) hydroxylamine (HA) and its derivatives are predominantly mutagenic for transforming DNA. This effect is caused by the direct reaction of HA with cytosine leading to base pair changes $\frac{G}{C} \rightarrow \frac{A}{T}$.

b) At low concentrations ($<$ 0.1 M), HA or its derivatives that have a free NOH group (N-methyl-HA, hydroxyurethan) exert a strong lethal effect on transforming DNA, whose rate remains constant when the HA concentration changes over several powers of 10.

c) The lethal effect is caused indirectly by a reaction product of HA and oxygen. The reaction is absent in the presence of 0.05 M pyrophosphate and it is quenched by reducing agents.

d) The product of HA and oxygen reacts with thymine, guanine, and uracil. The latter base can react with HA both directly and indirectly. The reaction is most drastically observed in the presence of HA by the slow melting of DNA far below the normal melting temperature. At the time at which the DNA strands separate completely (complete melting) about 50% of both thymine and guanine bases have reacted, as follows from the rate of the subsequent OD decrease.

2. Studies on chromosomes. The knowledge of the existence or absence of units linking DNA double strands together is very unsatisfactory. This stems mainly from experimental difficulties, since optimally peptides of about 2000 molecular weight would link DNA units of 5 million molecular weight. The high viscosity of DNA and the tendency of protein with basic groups to attach to DNA make a genuine identification of linking units a frightful undertaking.

At present, three facts have been cited as evidence for such links. a) Highly purified DNA still carries along certain peptides which might be covalently bound to it. b) When DNA is isolated by the most careful methods it has a lower molecular weight when it comes from thymus nuclei or bacteria than when it comes from T4 phages. c) Hydroxylamine causes chromosome breaks; this can no longer be regarded as evidence for DNA links because we have found that low concentrations of HA indirectly react with thymine and guanine, a fact which was not previously known.

Studies concerned with b) have been carried out by Dr. Richard Welsh, who is now a guest in our laboratory. He believes that he has found an enzymic fraction which, together with a so far unidentified substrate fraction, causes the polymerization of DNA pieces. The reaction depends on Mg^{++} and ATP and is observed by viscosity measurements and centrifugation.

Significance of Program to the Institute: Our findings explain how large chromosomal alterations can arise by chemical action. Hydroxylamine derivatives are normal metabolites and in some organisms (or persons) may not be degraded rapidly enough. If such a tendency of spontaneous chromosomal breaks should be observed in some persons, their tendency towards disease (in particular cancer) could be explained. It might then be possible to find agents, such as pyrophosphate, which reduce the chromosomal breaking effect.

Other cytotoxic agents probably induce chromosomal breaks similar to the hydroxylamines by first undergoing a reaction in the cell which gives rise to the active reagent.

The knowledge of the structure of chromosomes is important for the understanding of mutations, chromosomal duplication and function. In addition, these studies promise an explanation of repair mechanisms that have been cytologically observed after x-radiation damage.

Proposed Course of Project: 1. Determine whether the reaction product of HA can directly break the sugar phosphate backbone of DNA or whether the observed breaks arise indirectly via the alteration of the bases.

2. Investigate the lethal, mutagenic, and breaking effect of another class of important agents, the nitroso compounds.

3. Purify the active components of extracts that lead to the polymerization of DNA subunits.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Freese, E. B., and Freese, E.: Two separable effects of hydroxylamine on transforming DNA. Proc. Nat. Acad. Sci. 52: 1289-1297, Nov. 1964.

Freese, E.: The influence of DNA structure and base composition on mutagenesis. In Proceedings of the XI International Congress of Genetics, The Hague, The Netherlands, September 1963. Genetics Today. Oxford, England, Pergamon Press, 1964, pp. 297-306.

Freese, E. B.: The effects of urethan and hydroxyurethan on transforming DNA. Accepted for publication in Genetics, June 1965.

Honors and awards relating to this project: None

Serial No. NDB(I)-63 LMB/OC 1074
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Genetic Analysis of Virus-Induced Mutants of Escherichia coli.

Principal Investigator: Austin L. Taylor, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objectives: The objective of this study has been to employ genetic and enzymological methods of analysis in combination in an effort to elucidate the mechanism of mutation induction by phage Mu-1 of Escherichia coli. Experiments have been performed 1) to study the nature of the link between the prophage (latent virus) and the bacterial genome in virus-induced mutants, 2) to look for possible differences in enzymatic activity among virus-induced lactose negative (lac^-) mutants as compared to chemically induced lac^- mutants, and 3) to isolate the lactose messenger RNA for the ultimate purpose of detecting possible changes in the size of m-RNA derived from mutants which carry Mu-1 prophage in the lactose operon.

Methods Employed: Conventional methods of genetic analysis and of enzyme assay were employed in this study.

Major Findings: 1. Genetic studies. Most of the current work has been concerned with lactose negative mutants, mainly because so much is already known about the genetics, enzymology, and regulation of lactose metabolism in E. coli. Thirty different lac^- mutants were isolated from lac^+ cultures after infecting and lysogenizing these with the mutagenic phage Mu-1. The mutant frequency was roughly 10^{-3}, or about 100 times the frequency of spontaneous lac^- mutants in these strains. The analysis of

genetic recombinants issuing from crosses between the lac$^-$ mutants and non-lysogenic lac$^+$ parental strains showed that each mutant has a Mu-1 prophage genetically linked to the defective lactose operon. The linkage was absolute in that no recombination between the lac$^-$ phenotype and the prophage could be demonstrated. In partial diploids of the genotype lac$^-$ (Mu-1)$^+$/lac$^+$ (Mu-1)$^-$, the lac$^-$ trait is always recessive to the wild type allele; thus only those lactose genes which are *cis* to the prophage are inactive. This rules out the possibility that prophage might inactivate the lactose operon by means of a cytoplasmic repressor.

Through a series of genetic manipulations, stocks were prepared which would allow a determination of the frequency of recombination between closely linked genes on either side of the lactose operon, both in virus-induced lac$^-$ mutants and in wild type lac$^+$ bacteria. These experiments showed that the recombination frequency between the outside markers was significantly greater in the lac$^-$ mutants than in the wild type strains. This indicates that the chromosomal distance between the outside markers is greater in the mutants than in wild type bacteria, and this is taken as evidence that the viral genome is physically inserted into the lactose operon in these mutants. A similar loosening of genetic linkage between outside markers was also demonstrated in another part of the *E. coli* chromosome, namely the threonine-pyrimidine A-leucine region.

2. *Biochemical studies*. Cell-free crude extracts were prepared from each of the 30 lac$^-$ mutants described above. In addition, extracts were prepared from wild type bacteria and from 19 different lac$^-$ mutants isolated from non-lysogenic cultures treated with ultraviolet light or N-methyl-N-nitro-N-nitrosoguanidine (NG) as mutagenic agents. The extracts were assayed for the two enzymes which are determined by the lactose operon and which are amenable to *in vitro* assay: β-galactosidase and β-galactoside transacetylase. Of the 19 UV or NG-induced mutants, 15 were defective in β-galactosidase synthesis; 11 of these had measurable partial β-galactosidase activity and transacetylase activity ranging from partial to normal, while 4 were polarity mutants completely devoid of both enzyme activities. The remaining 4 mutants had normal β-galactosidase levels and were presumably deficient in lactose permease; three of these had normal transacetylase levels and one had no transacetylase activity.

Of the 30 virus-induced mutants, 17 were strong polarity mutants having no measurable amounts of either enzyme assayed. The remaining 13 mutants had normal levels of β-galactosidase, but no measurable transacetylase activity. These results show that the Mu-1 phage can selectively attack one part or another of the lactose operon, but that wherever the attack occurs, the resulting genetic lesion is a severe one that leads to a complete shut-down of the transacetylase gene which is located furthest from the "operator" or beginning end of the operon. The severity of the

virus-induced lesions, as compared to UV or NG-induced mutations, can perhaps be best explained on the hypothesis that viral DNA is directly inserted into the structural genes of the host bacterium.

If correct, this model would predict that messenger RNA derived from a lactose operon containing an inserted prophage might be considerably larger than the normal messenger. The search for lactose m-RNA, in the form of polyribosomes having associated β-galactosidase activity, is still at a preliminary stage and has not yet produced useful results. The main obstacle to further progress is the absence of suitably gentle methods for extracting intact polyribosomes from whole cells. The development of such methods is still in progress.

Significance to Program of the Institute: The results obtained in the past year have brought us considerably closer to understanding the mechanism by which a foreign genetic element, or episome, can induce heritable modifications of the host genome. In recent months, several other investigators have attributed a variety of genetic alterations in bacteria to the action of hypothetical episomic elements. The model system under investigation here should continue to provide more detailed information on the mechanism of such episome-mediated hereditary transformations.

Proposed Course of Project: Principal emphasis will be given to a continuing study of virus-induced lac$^-$ mutants. In particular, experiments will be designed 1) to isolate and characterize lactose polyribosomes in such mutants, 2) to search for possible interactions between regulatory functions of the lactose operon and regulatory functions of prophages associated with this operon, and 3) to study the genetic fine structure of lac$^-$ mutants for the purpose of determining the number of sites of prophage integration within the individual structural genes.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Taylor, A. L., and Thoman, M. S.: The genetic map of Escherichia coli K-12. Genetics 50: 659-677, October 1964.

Taylor, A. L., Beckwith, J. R., Pardee, A. B., Austrian, R., and Jacob, F.: The chromosomal location of the structural gene for orotidylic acid pyrophosphorylase in Escherichia coli. J. Molec. Biol. 8: 771, May 1964.

Honors and awards relating to this project: None

Serial No. NDB(I)-65 LMB/OC 1244
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Control and Evolution of Protein Synthesis and Function

Principal Investigators: Michael Cashel, M.D., and Ernst Freese, Ph.D.

Other Investigators: Michael Kaback, M.D., Harry Taber, Ph.D. (Guest Worker), and Richard Wax, Ph.D.

Cooperating Units: None

Man Years:

Total:	4.3
Professional:	3.1
Other:	1.2

Project Description:

Objectives: The control mechanisms involved in sporulation and germination provide a typical and especially simple example of differentiation. So far we have directed our work toward the elucidation of germination and, in particular, the role of alanine dehydrogenase (AlD) in germination. AlD is also being used as a model for artificial enzyme evolution and for the control of enzyme induction. Experiments concerned with the control of sporulation have also been initiated. As side projects, some results on the lysis of bacteria and the properties of their cell walls have been obtained.

A portion of this project is a partial continuation of Project NDB(I)-62 LMB/OC 948.

Methods Employed: Spores of Bacillus subtilis were obtained on a specially designed minimal as well as a complete medium. They were purified by polyethylene glycol phase separation, cesium chloride centrifugation, and 10 x washing with water. The frequency of refractile spores was determined in the phase contrast microscope; it was always > 90% for the spore employed. Germination was measured by the decrease of OD_{625} and followed microscopically. Ca^{45} containing spores were made by growth in Ca^{45} and purification as described above.

AlD$^-$ and other mutants were isolated by strong mutagenic treatment either of bacteria with ethyl methane sulfonate or of spores with γ-rays. The mutant properties were transferred to other bacteria by transformation. AlD and other enzyme activities of normal and mutant strains were measured spectrophotometrically by routine methods described in the literature.

For the alkaline phosphatase experiments bacteria were grown in minimal-glucose medium containing limiting amounts of 1×10^{-4} M. Phosphate, alkaline phosphatase, AlD and lactic dehydrogenase activity were determined at different times throughout growth period.

For the lysozyme lysis experiments, both gram positive and gram negative bacteria were employed. Stock suspensions contained stationary phase bacteria which are normally resistant to lysozyme lysis. Protoplast studies were performed using B. subtilis only.

Major Findings: 1. Changes in alanine dehydrogenase activity often, but not always, are associated with impairment of sporulation and pigment formation. A deficiency in alanine dehydrogenase may or may not appreciably alter normal mechanisms of germination. These observations suggest the presence of another enzyme(s), which in addition to alanine dehydrogenase may involve a step in germination as well as initial stages in sporulation. Preliminary studies on mutants lacking the ability to convert α-aminobutyric acid to α-ketobutyric acid appear to be consistent with this hypothesis: when such mutants also lack alanine dehydrogenase they appear to always lack germinating ability in the presence of either alanine or α-aminobutyrate.

A genetic analysis of these mutants will enable us to decide which enzymic properties are strictly correlated with germination. The properties of the α-aminobutyrate deaminating enzyme are also of further interest because this enzyme is not required for vegetative growth but seems to be needed for certain steps in spore differentiation. In addition, both this enzyme and AlD may be under common control implicating a general molecular control mechanism affecting multiple steps in differentiation. (Cashel, Wax, Freese)

2. We intend to isolate mutants of AlD which have an altered substrate specificity. Such mutants are of twofold interest. On the one hand, they prove that artificial evolution is feasible on the molecular level and that therefore new enzymes, not occurring in nature, can be artificially developed. On the other hand, such a mutant might also be altered in the mechanism controlling AlD synthesis; this finding would prove that the enzyme would be involved in its own control.

The most promising substrate for the isolation of AlD with altered substrate specificity seems to be α-aminobutyrate. As a first condition for its utilization as a selective agent we had to isolate mutants that

would not utilize this substrate for any other biochemical step. Such mutants have now been obtained, and the selection of new enzymes is under way. (Wax)

3. Nascent proteins are bound to ribosomes together with messenger RNA. It may be possible to isolate this complex by its attachment to antibodies. To this end we have isolated crystalline AlD and immunized rabbits. For the quantitative and yet specific examination of antigen antibody precipitation we are using a micromethod with capillaries. The antibodies will also be used for the identification of mutant AlD^- proteins. (Kaback)

4. Several Krebs cycle and respiratory (cytochrome C) enzymes change their concentration during growth and sporulation of B. subtilis. They are presumably controlled by a common mechanism which we shall attempt to uncover.

Instruments have been obtained for the measurement of Krebs cycle enzymes and for the rapid spectrophotometric determination of cytochromes in bacterial mutants. Activity measurements of normal and mutant bacteria are under way. (Taber)

5. It was discovered accidentally that the addition of $MgCl_2$ to stationary phase cells which had been incubated at 37° in lysozyme resulted in instantaneous lysis of B. subtilis cells, whereas in the absence of $MgCl_2$ lysis did not occur. Examination of effects of various compounds instead of $MgCl_2$ revealed that only cationic species were effective, divalent ions were in general more effective than monovalent ions, and that Mg^{++} or Mn^{++} were the most effective of a variety of divalent cations studied. Measured spectrophotometrically, the degree of lysis after addition of 0.05 M $MgCl_2$ is proportional to the exposure time of cells to lysozyme for intervals less than 5 minutes and is also inversely related to cell concentration. Studies on the effect of varying lysozyme concentration reveal that partial lysis will occur when stationary cells are incubated with very low lysozyme concentration (1-5 μg/ml), even in the absence of $MgCl_2$. Nevertheless, the addition of $MgCl_2$ enhances lysis even under these conditions. In the presence of higher lysozyme concentrations, no lysis is observed unless $MgCl_2$ is added. No lysis of protoplasts or whole cells was observed in isotonic sucrose after $MgCl_2$ addition. Lysis by addition of Mg^{++} to lysozyme treated cells was observed for Escherichia coli, Clostridia kluyveri, and Bacillus subtilis, but not for Aerobacter or Serratia.

Since protoplasts in sucrose cannot be broken by salt addition, the observed lysis must be connected with the bacterial cell wall; two mechanisms may be involved. On the one hand, triple concentrations of lysozyme may cover the bacterial cells preventing their lysis. On the other hand, the teichoic acid structures of bacteria which are insensitive to lysozyme

may be held together by divalent cations. In either case addition of an excess of cations obviously breaks the protecting complexes. A simple technique is now available for extremely rapid lysis of bacteria which is advantageous for cell enzyme studies. The method would be of value in extraction of labile compounds such as polyribosomes, RNA, RNA polymerase, etc. (Cashel and Kaback)

6. Working with different bacteria it appeared surprising to us that in B. subtilis certain enzymes were released (exoenzymes), whereas in E. coli the corresponding enzymes were retained but could be liberated by lysozyme treatment (perienzymes). This suggested a principal difference between the two types of bacterial cell walls. If this assumption was correct one would predict that perienzymes in E. coli should be exoenzymes in B. subtilis and vice versa. We have verified this assumption for alkaline phosphatase.

When B. subtilis was grown in minimal-glucose medium containing limiting amounts of phosphate, alkaline phosphatase became derepressed after a certain time of growth. In contrast to E. coli, the enzyme was secreted into the medium as soon as activity could be measured. Two other enzymes, however, alanine- and malic-dehydrogenase remained inside the bacteria. These results agree with our expectation, since alkaline phosphatase is a perienzyme in E. coli, whereas the other two enzymes cannot be released even by protoplasting treatment. (Cashel and Freese)

Significance to Program of the Institute: The molecular understanding of functional changes in biological systems produced by alterations of protein structure or by derangements of the mechanisms of control of enzyme synthesis is more amenable to analysis in microorganisms than in higher organisms. Nevertheless, fundamental biologic processes are essentially the same in microbes as in higher organisms. As a result, relatively simple models of more complex systems may be isolated and a genetic and biochemical understanding more readily obtained. Clarification at this level is therefore a prerequisite to the understanding of normal and disease processes.

Sporulation and germination in Bacillus subtilis is a model system for the study of differentiation. As determined by studies with enzyme deficient mutants, it has been established that one enzyme, alanine dehydrogenase, has unique developmental significance for the organism. In contrast, a deficiency of this enzyme has no effect on normal growth properties. In addition, a second enzyme which converts α-aminobutyrate to α-ketobutyrate appears necessary for initiating the process of germination. This suggests a single control mechanism affecting enzyme systems involved in critical steps in differentiation.

Hereditary diseases may be classified in two categories: a) alterations in structure of the gene products, and b) alterations in the

mechanisms of control of synthesis of normal gene products. In both categories the functional activity of the enzyme is diminished or altered. We have developed model systems in microorganisms which will allow study of prototypes of both categories. We have also developed a model system for the study of evolution of protein structure as related to specificity of enzyme substrates.

Using immunological methods, an attempt is being made at dissecting out of the cell the component structures involved in protein synthesis. If successful, such a method holds promise for not only a more fundamental understanding of protein synthetic mechanism, but also for isolating messenger RNA specific for one enzyme, alanine dehydrogenase.

Gene products ultimately determine cellular structure, for example cell walls and membranes. An extremely rapid and gentle means of disrupting cell walls of some bacteria has been found which may allow isolation of labile cellular components. In addition, it may give insight into molecular processes which are intimately associated with these structures. Cellular excretion of enzymes into the environment has been shown to be a species' specific function of the cell wall.

Proposed Course of Project: Using genetic and biochemical methods, we shall attempt to further elucidate critical steps in the germination-sporulation sequence as well as investigate common mechanisms of control and evolution of protein synthesis and function.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Cashel, M., and Freese, E.: Initial stages of germination. In Campbell, L. L., and Halvorson, H. O. (Eds.): Spores III, A Symposium held at Allerton Park, Ill. 2-4 October, 1964. Ann Arbor, Mich., American Society for Microbiology, 1965, pp. 144-151.

Cashel, M., and Freese, E.: Excretion of alkaline phosphatase by Bacillus subtilis. Biochem. Biophys. Res. Commun. 16: 541-544, August 1964.

Honors and awards relating to this project: None

Serial No. NDB(I)-62 LMB/OC 948
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Comparison of Structure and Function of Three Dehydrogenases of Bacillus subtilis.

Principal Investigator: Akira Yoshida, Ph.D.

Other Investigator: Ernst Freese, Ph.D.

Cooperating Units: None

Man Years:

Total:	2.1
Professional:	1.1
Other:	1.0

Project Description:

Objectives: 1. Isolation and crystallization of alanine dehydrogenase (AlD), malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) from B. subtilis. 2. Elucidate their molecular size, subunits molecular size, amino acid composition, serological characteristics and enzymic properties. 3. Discover their evolutional relationships on the basis of their structural, serological and functional similarity.

Methods Employed: Characterization of molecular structure. Amino acid composition and molecular weight were determined by automatic analyser and by sedimentation equilibrium method. Subunit molecular weights were estimated from 1) patterns of fingerprinting of tryptic peptides and lysine and arginine content of the proteins, and 2) patterns of sedimentation equilibrium of the reduced, denatured and dissociated proteins in guanidine-HCl.

Serological characteristics. Using rabbit anti-serum against the individual dehydrogenase, their serological properties were examined by 1) precipitin tests in agar gel plates (double diffusion) and in capillary tubes, 2) complement fixation tests, 3) neutralization of the enzyme activity, and 4) passive cutaneous anaphylaxis tests.

Major Findings: Although the three dehydrogenases are different with respect to their molecular size, amino acid composition and enzymic specificity, they showed significant similarities, described below, which would indicate that they have common (or closely similar) peptide portions in their molecules and that they might be developed from a common ancestor in the process of evolution.

1. Serological cross reaction was observed between AlD and MDH, and between MDH and LDH (that between AlD and LDH being weaker).

2. AlD (MW = 228,000) is composed of six identical subunits, MDH (MW = 148,000) is composed of four identical subunits, and LDH (MW = 146,000) is composed of four identical subunits. The amino acid composition of these subunits differ but their molecular weights are approximately the same.

3. AlD and MDH have at least four fingerprinting spots in common, and LDH has two of these spots in common.

4. The three enzymes are closely similar with respect to the availability of NAD and NAD analogs as substrates.

Significance to Program of the Institute: An understanding of the relationships of molecular structure and function of such evolutionally related enzymes as AlD, MDH and LDH can be related to an understanding of various diseases which are associated with hereditary modification of proteins.

Proposed Course of Project: This project is complete. Amino acid sequence of common peptide portions of the three enzymes will be elucidated, and the structure of the peptide regions which are implicated in their function will be studied under Project NDB(I)-65 LMB/OC 1244.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Yoshida, A., and Freese, E.: Enzymic properties of alanine dehydrogenase of Bacillus subtilis. Biochim. Biophys. Acta 96: 248-262, February 1965.

Yoshida, A.: Purification and chemical characterization of malate dehydrogenase of Bacillus subtilis. J. Biol. Chem. 240: 1113-1117, March 1965.

Yoshida, A.: Enzymic properties of malate dehydrogenase of Bacillus subtilis. J. Biol. Chem. 240: 1118-1124, March 1965.

Yoshida, A., and Freese, E.: Purification and chemical characterization of lactate dehydrogenase of Bacillus subtilis. Accepted for publication in Biochim. Biophys. Acta.

Yoshida, A.: Enzymic properties of lactate dehydrogenase of Bacillus subtilis. Accepted for publication in Biochim. Biophys. Acta.

Yoshida, A.: Structural and serological similarity of three dehydrogenases of Bacillus subtilis. Accepted for publication in Biochim. Biophys. Acta.

Yoshida, A.: Micro method for the quantitative determination of L-alanine. Accepted for publication in Anal. Biochem.

Honors and awards relating to this project: None

Serial No. NDB(I)-65 LMB/OC 1208
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Participation of Ribonucleic Acid in the Regulation of Cellular Metabolism.

Principal Investigator: Robert A. Lazzarini, Ph.D.

Other Investigators: Elisabeth Bautz Freese, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.5
Other:	0

Project Description:

Objectives: Currently a body of evidence is developing that indicates that the control of protein synthesis is exerted at the level of both messenger RNA and transfer RNA. Although the role of transfer RNA in synthetic reactions is well established, the details of its role in regulatory mechanisms are unknown. The primary objective of this project is to study the participation of RNA, particularly transfer RNA, in the regulation of protein synthesis and gross morphogenic changes in Bacillus subtilis.

Methods Employed: In addition to standard biochemical techniques, column chromatography on methylated albumin coated kieselguhr has been used to purify and fractionate various species of transfer RNA. Heterologous amino acid activating enzymes are used to estimate the heterogeneity within a particular species of transfer RNA and the polyribonucleotide directed binding of transfer RNA to ribosomes is used to measure the relative amounts of the degenerate forms of transfer RNA.

Major Findings: Work on this project has just commenced and major findings have not as yet been achieved.

Significance to Program of the Institute: Control of protein synthesis and of the grosser morphogenic changes (sporulation and germination) in bacterial systems represents examples of regulatory mechanisms which are at this juncture more amenable to experimental investigation than the more specialized mammalian systems by virtue of the number of parameters which can be varied and controlled easily. It is anticipated that information obtained from these simpler systems will help form a basis upon which to investigate the more complex regulatory mechanisms operative in higher organisms and eventually to understand and control disease states of these systems.

Proposed Course of Project: The initial phases of this project will involve 1) a detailed analysis of the micro heterogeneity and degeneracy of various species of transfer RNA isolated from vegetative, sporulating and germinating cells of B. subtilis; 2) a comparison of those forms of transfer RNA that are capable of being esterified with amino acids in vivo with those that accept amino acids during the in vitro esterification with amino acids. It is anticipated that the results of these studies will establish whether or not some of the multiple forms of transfer RNA are altered or otherwise made unavailable to the protein synthesizing machinery during gross morphogenic changes.

Part B included: No

Serial No. NDB(I)-62 LMB/OC 945
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Large Volume Synchronization of Bacterial Cell Division.

Principal Investigator: Donald J. Cummings, Ph.D.

Other Investigators: None

Cooperating Units: Instrument Engineering and Development Branch, DRS

Man Years:

Total:	0.4
Professional:	0.3
Other:	0.1

Project Description:

Objectives: In the project report of 1963, a technique for obtaining $4\text{-}5 \times 10^8$ cells/min in synchrony was described. The method used was the filtration of exponentially growing bacterial cells onto millipore paper, diameter 293 mm, subsequent inversion of that paper and elution with minimal medium. After about 40 minutes of elution, the bacteria coming off the millipore were the youngest cells in an exponential population and these cells proceeded to grow synchronously for at least three generations. The present report will describe the results obtained on Escherichia coli B/r with respect to DNA, RNA and β-galactosidase.

Methods Employed: The synchronization procedure was the same as previously described. DNA was measured by the Burton diphenylamine method, RNA by the Mejbaum orcinol technique and β-galactosidase was induced and assayed by the method of Pardee, Jacob and Monod.

Major Findings: It was found that DNA and RNA were synthesized continuously throughout the generation cycle of a synchronously growing population of E. coli B/r. The stepwise versus continuous synthesis of these macromolecules has been disputed for some time and the present

results indicate that in B/r, the synthesis is indeed continuous, as has been shown by pulse labeling with N^{15}.

The synthesis of β-galactosidase was also continuous. Moreover, this continuous synthesis could be stopped at any time by the addition of chloramphenicol or mitomycin C. The cessation of synthesis occurred almost immediately with chloramphenicol and with a lag of 20 minutes in the case of mitomycin C. These results were related to the involvement of a messenger RNA specific for β-galactosidase.

Significance to Program of the Institute: Very little is known about the sequence of events during the division cycle of bacteria since little information can be obtained from a single cell. The promise of a large number of cells all in the same stage of the division cycle opens unlimited opportunities for biochemical and genetic studies. Synchronously dividing populations can be used not only to observe when a cellular process occurs, but also how it occurs by following its course through the division cycle.

Proposed Course of Project: This project is complete.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Cummings, D. J.: Macromolecular Synthesis during Synchronous Growth of Escherichia coli B/r. Biochim. Biophys. Acta 95: 341-350, Feb. 1965.

Honors and awards relating to this project: None

Serial No. NDB(I)-62 LMB/OC 946
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Structural Features of the T-Bacteriophages

Principal Investigator: Donald J. Cummings, Ph.D.

Other Investigators: None

Cooperating Units: Laboratory of Neuroanatomical Sciences, NINDB
(Dr. Milton Brightman)

This project is complete.

Serial No. NDB(I)-63 LMB/OC 1069
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: In situ Organization of Bacteriophage DNA.

Principal Investigator: Donald J. Cummings, Ph.D.

Other Investigators: T. Wanko, M.D.

Cooperating Units: Ophthalmology Branch, NINDB

This project is complete.

Serial No. NDB(1)-63 LMB/OC 1072
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Role of Small Molecular Weight Ribonucleic Acids in Biological Systems, their Isolation and Chemistry: A Mild Chemical Method for the Removal of a Terminal Nucleoside from RNA and the Identification of the Nucleoside as its Free Base

Principal Investigator: David H. Rammler, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: In concert with the general program of this laboratory on the chemistry of RNA, it was necessary to find a method for removing the terminal nucleoside from an RNA molecule with minimum alterations with regard to the remaining molecule. Over the years considerable information on periodate oxidations of ribonucleic acids has accumulated; however, no cogent mechanism for this reaction has been proposed. We have elucidated the chemical mechanism of this reaction.

Methods Employed: Standard chemical techniques.

Major Findings: Periodate oxidation of ribonucleosides and ribonucleotides in the presence of amines requires 4 moles of periodate per mole of nucleoside. The initial oxidation occurs at the cis glycol pair. A subsequent second oxidation is dependent on the formation of a Schiffs base with the amine present. This oxidation is non-specific and occurs rapidly at 37°, pH 7.0. The second non-specific oxidation utilizes 3 moles of periodate per mole of nucleoside and yields in addition to glycoaldehyde

phosphate, in the case of ribonucleotides, 2 moles of formic acid, 1 mole of carbon dioxide, and the free base obtained from the nucleoside or nucleotide. The total amount of free base released was found to be less than the theoretical amount by about 12%. By using a nucleotide which was specifically labeled in the purine nucleus (adenosine-5' phosphate), we were able to demonstrate that the 12% loss in recovery of the base could be accounted for by hydrolysis of the purine nucleus. This mechanism explains the anamolous over-oxidations originally found in the structure proofs for amino-nucleosides such as puromycin. The method is sufficiently mild so that end nucleosides of RNA viruses can be determined. Studies of this type were carried out with the RNA virus Ms-2. The results indicated that the viral RNA as isolated by the present methods is considerably heterogeneous with regard to end nucleosides.

Significance to Program of the Institute: Information obtained on methods for the structural analysis of ribonucleic acids are of considerable importance in the problem of correlating structure and function of RNA in biological systems.

Proposed Course of Project: This project is complete. A paper for publication has been submitted to Biochemistry.

Part B included: No

Serial No. NDB(I)-63 LMB/OC 1073
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Role of Small Molecular Weight Ribonucleic Acids in Biological Systems, their Isolation and Chemistry: Chemical Alterations of Polyuridylic Acid and its Effect on the Incorporation of Phenylalanine in a Cell Free Protein Synthesizing System.

Principal Investigator: David H. Rammler, Ph.D.

Other Investigators: Creed Abell, Ph.D. and A. Delk

Cooperating Units: Carcinogenesis Studies Branch, NCI
Serial No. 4544

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: As part of the program in this laboratory on the chemistry of ribonucleic acids, it was of interest to attempt to chemically modify the structure of RNA. Using polyuridylic acid as a model, various solvent conditions necessary to carry out specific reactions were tested to examine their effects on the integrity and biological activity of polyuridylic acid.

Methods Employed: Using the cell free amino acid incorporating system described by Nirenberg as an assay for biological activity, we followed the loss in biological activity after treatment with various solvents with alterations in structure as evidenced by chemical and physical methods.

Major Findings: Considerable loss in biological activity, 80-90%, is observed after treatment of polyuridylic in 0.01 M acetic acid at 37° for 1 hour. Similar losses occur with other buffers at this pH. The loss in activity is dependent on ionic strength and is directly proportional to the concentration of the polymer in solution.

Significance to Program of the Institute: Information obtained on the chemical properties of polyuridylic acid and the effect of chemically altering the structure of polyuridylic acid on its ability to direct phenylalanine incorporation in cell free amino acid incorporating systems is of considerable interest.

Proposed Course of Project: This project is complete. A paper has been submitted for publication in Biochemistry.

Part B included: No

Serial No. NDB(I)-63 LMB/OC 1070
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Role of Small Molecular Weight Ribonucleic Acids in Biological Systems, their Isolation and Chemistry

Principal Investigator: David H. Rammler, Ph.D.

Other Investigators: Lawrence R. Fowler, Ph.D. and Carmen Grado, Ph.D.

This project is complete.

SUMMARY REPORT
July 1, 1964 to June 30, 1965
Laboratory of Perinatal Physiology
National Institute of Neurological Diseases and Blindness

Ronald E. Myers, Chief

In October 1964, the Laboratory of Perinatal Physiology began functioning under a new directorship. As a consequence, some activities of the Laboratory have been curtailed and other areas of interest are in the process of development. Much of the summary report will be given over to discussion of areas of interests which will characterize the new program of the Laboratory.

One of the most unique and interesting facilities of the Laboratory of Perinatal Physiology, historically and scientifically, has been the free-ranging monkey colonies. The activities of the Section of Primate Ecology involved with the field studies have been centered on three small offshore islands, Cayo Santiago, La Cueva, and Guayacán. Cayo Santiago, at the eastern end of Puerto Rico, was first established as a facility containing free-ranging monkey groups in 1938 with the placing of 409 monkeys on the island for studies of reproductive behavior. In the intervening years and until 1956, the numbers and fortunes of the monkeys on this island waxed and waned. In 1956 when the National Institutes of Health initiated studies of the monkey groups, only 150 monkeys remained. In 1961, the islands of La Cueva and Guayacán, located together at the western end of Puerto Rico, were developed by the Laboratory into a second important field station for comparative studies of reproductive cyclicity and for study of primate behavior. On these three islands has been established a wide-ranging and significant program for studies of population dynamics, reproductive cyclicity, establishment and dissolution of bands, social and reproductive behavior, maternal and child behavior, and many other valuable studies. The singularity of these field facilities derives from the fact that the monkey social groups studied have evolved in an undisturbed natural circumstance for three decades. The resultant social organizations, therefore, are believed to reflect most closely the multitude of factors which direct the evolution of the social phenomenon. The studies which have been carried out in the past and which will continue have been studies of free-ranging social bands and descriptions of behaviors of individuals within these bands. The sizes of the islands are compatible with free movement of the several individual bands which constitute the populations.

Important contributions to our knowledge of the primate situation from the studies of the Section on Primate Ecology include the following:

1. Monkeys in a free-ranging circumstance exhibit an annual reproductive cyclicity with a well defined 3-4 month breeding period in the Fall, and a similar 3-4 month

period in early Spring of infant births.

2. The annual reproductive success of adult free-ranging female monkeys approaches 86 per cent compared to 43 per cent in the caged colony in San Juan.

3. The free-ranging monkey bands exhibit very small to vanishing incidences of abortion and infant loss at the time of parturition in contrast to high incidences in the San Juan caged colonies.

4. Individual social bands on the islands have in a few instances split into two bands. This process is not related to overall band size, but is a reflection rather of complex interactions and relations between more dominant individuals within the bands.

5. The artificial aggregation of animals for extended periods in close confinement is not a sufficient condition for band formation on release. Rather, other factors not yet defined seem to play primary roles in band formation.

6. There exists a remarkable long term stability in the identity and inter-relationships expressed between individuals within social bands.

7. Leadership status in social bands seem frequently related to the social status of the mother. Leaders of bands are found to be male offspring of dominant females.

8. The interindividual regulation and control of behavior within social bands and between individuals of different bands is characterized by aggressive displays and posturing to a vastly greater degree than actual involvement in physical conflict and fighting.

9. Despite the conspicuous aspect of aggressive displays, positive encounters between individuals of a band such as grooming and physical association are quantatively far more prominent.

In addition to these examples of specific insights into the reality and expression of primate social behavior, there has been a rich flow of normative data descriptive of mother-infant relationships, sibling interactions, male-female behavior during and outside of the breeding season, hierarchical organization within the monkey bands, characterizations of such social acts as grooming, mounting, aggressive displays, displacement activities, and other manifestations of communication and interaction within the social groups.

The Laboratory of Perinatal Physiology hopes to develop an Experimental Compound Facility in the San Juan area for the experimental analysis of social and reproductive behavior. Experimental approaches represented may be telemetry, endocrinological manipulation by organ extirpation or hormone

implantation, manipulation of experiential or psychological variables, and the ablation of brain tracts or brain centers. An assumption underlying many of the anticipated studies is that experimental procedures carried out on the individuals, whether endocrinological, neurosurgical, or experiential should be carried out during infancy rather than during adult life in order to explore the full importance of the factors varied on the development and sustenance of function. Specific brain lesions or other manipulations will, therefore, be carried out in small populations of infant monkeys. Thereafter, these infant populations will be placed in enclosures to develop and express their own specific social organizations. The social orders so evolved will be compared to control groups of monkeys raised under similar circumstance but without experimental manipulation. Such studies should contribute to the solution of some of the most central problems of social behavior and social organization. The enclosure facility will also enable meaningful experimental studies to be initiated and carried out on such problems as maternal behavior, maternal-infant relationship, infant-sibling interactions, male-female relations, communication functions in primate social groups, etc.

Within the central laboratory in San Juan, studies will be carried out in several areas of concern as follows:

1. Physiological Psychology - The main concern will be definition of neural mechanisms underlying perception, memory and learning. Past efforts have been devoted primarily to studies of vision and touch. Future work will continue along the same lines and will be extended to studies also of audition and olfaction. This will allow for cross-comparisons between the patterns of organization and functions of the several sensory perceptual systems. The approach emphasized is utilization of formal behavioral training and testing situations which enable the investigator to tie down the perceptual, memory and learning processes, and to express them in terms of learning curves, levels of performance, and in terms of discriminative capacity attainable. The effects upon these functions of various types of brain lesions and of brain stimulations both in cortex and in subcortical mechanisms, can then be defined. Efforts will continue in the direction of defining the capacities of these systems both as intact and impaired mechanisms in the functions of information handling and memory development.

Also included within the area of physiological psychology will be efforts directed toward an understanding of the physiological basis for the perceptual functions utilizing the techniques of modern electroneurophysiology. Explorations within this context will include investigations of the functional characteristics of neuronal populations and systems which underlie and support perceptual and mnemonic functions. They will include studies of aberrations of normal function resulting from aberrations in development such as occur in the disease amblyopia ex anopsia. They will include also studies of the development of normal function as expressed during intra and early extrauterine existence.

2. Comparative Neurology - The section on comparative neurology will be concerned with definitions of the patterns of overall organization of the nervous system of the monkey using experimental neuroanatomical techniques. Specific functional systems and neural centers will also be separately

studied in an effort to determine the details of intrinsic organization or interconnection within the system. Answers as to the identity and nature of anatomical interrelations between functional systems also will be sought. A comparative approach will also be used to the extent possible to give insight into phylogenetic trends in brain organization. This approach will attempt to (1) identify and describe patterns of overall brain organization and (2) to specify the anatomical interrelationships of the various mechanisms of the brains of select species of animals within the several classes of vertebrates. It is believed that such comparative studies will give rise to fundamental understanding of structural and functional homologies within the vertebrate series and will supply important clues as to the pattern of organization and the functional import of the brain centers of the nervous system of man himself.

3. Developmental Neurology - The availability of significant numbers of monkey pregnancies per year places the Laboratory of Perinatal Physiology in a most favorable position for the study of nervous system development. It is hoped, morphological and functional studies may be initiated directed toward furthering our understanding of the relation between structure and function. Particular attention should be paid to the newer techniques which offer the potential of greatly expanding our knowledge of developmental processes.

4. Experimental Neuropathology - The concerns within the area of experimental neuropathology are threefold. 1) To define the patterns of neuropathological change induced in the nervous system with various types of asphyxial and anoxic insults. These studies will concentrate on oxygen deprivation effects on the fetus in utero during gestation and at the time of birth. Studies will also explore anoxic CNS damage in the adult. 2) Definition of the mechanisms through which the various patterns of neuropathological change are induced in the nervous system. These studies will concentrate on alterations in cardiovascular system dynamics testing the assumption that the vascular system represents the weak link in the biological mechanism in response to anoxia. 3) Studies of alterations in the metabolism of single cells of the nervous system under conditions of anoxia. Attempts will be made to relate the alterations in the metabolism and biochemistry of the cells to the production of reversible and irreversible changes in morphology and function that result from oxygen deprivation.

During the past year the progress of studies in the Laboratory has been impaired by an attenuation of programs due to the hiatus in the directorship of the Laboratory. Many farreaching decisions concerning development of programs and replacement of professional staff were delayed pending appointment of a new chief of the Laboratory. At the present time the professional vacancies are in the process of being filled and it is hoped that in due course the program will again gain the strength which characterized the Laboratory in the past.

The Section on Neuropsychology has been involved in studies of development of the infant rhesus monkey. Investigations have been carried out to determine whether there is a period of increased impressionability or receptiveness to learning comparable to imprinting in birds. Studies of

conditioned avoidance in the infant monkey has revealed no evidence for such a distinct period of impressionability. Rather, following birth there is an initial two or three days of increased responsiveness to sensory stimulation in general followed by more normal levels of responsiveness. In regard to learning, the infant monkey during the first four days does not evidence signs of learning conditioned avoidance. Thereafter, however, learning occurs and the capacity for learning gradually increases into early juvenile existence. Differences are seen between C-sectioned and vaginally delivered infants in that the former exhibit decreased general responsiveness to sensory stimulation during the first five weeks of life. In addition, the C-sectioned animals exhibit depressed capacity for acquisition of conditioned avoidance during the 90 days in which learning has been studied.

A beginning has been made in studying the effects of brain lesions on the patterns of vocalization, general activity, and on the capacity for conditioned avoidance learning in the infant monkey. Lesions in the inferior colliculus and also sham operations prepared with needle penetration through posterior regions of the cerebrum have both resulted in decreased capacity for conditioning at all ages studied up to 90 days. General activity levels and vocalization were not affected by the lesions, but were dependent upon mode of delivery. Beginning studies have been carried out on effects of neocortical commissure section on these functions. Animals with commissure section exhibit lower scores at the onset of conditioned avoidance learning, but end up with significantly better scores than do normals or other animals tested, i.e., conditionability or capacity to learn the conditioned avoidance was enhanced after neocortical commissure transection.

Studies of sleep patterns of infant monkeys have continued in the past year. Sleep in the infant monkeys is characterized by stages similar to those of the adult. Both ages exhibit three overall states of vigilance normally: awakeness with normal alert activity, high voltage slow sleep associated with motor quietness, absent eye movements, and active electromyogram, and low voltage fast sleep associated with motor quietness, a low rate of discharge of the electromyogram and rapid eye movements. The amounts of time spent in these different phases is as follows: The percentage of sleep time spent in the low voltage fast phase rises from a low at the time of birth to an intermediate level during the first two days. During the subsequent five days there is a leveling off. Thereafter, a gradual increase is seen through the following twelve months. The overall time spent in any type of sleep is low at the time of birth, increases fairly rapidly to seven days, and, thereafter, again undergoes a gradual decline through the following twelve months.

Studies of sleep deprivation in early infancy, has revealed no post-deprivation alterations in the quantities of time spent in different types of sleep. In juveniles, however, after deprivation of either type of sleep there is a compensatory increase in the proportion of time spent in that type enduring several days after the end of deprivation. For the juvenile, and less so for the infant monkeys, as the period of deprivation was prolonged, there was increasing difficulty interrupting the specific type of sleep being deprived. This was noted in the frequency with which interruption was necessary and also in the current levels required to bring the animal out of the phase of sleep involved.

Animals have been studied with occluders over the eyes placed at the time of birth. Despite visual deprivation, the eye movements during low voltage fast sleep compared to visually normal animals. However, over the first six months of existence, there is a very gradual decline in the amplitude, the time rate of change, and the frequency of occurrence of the eye movements. By six months of age the deprived animals exhibit significant decreases in occurrence of eye movements during low voltage fast sleep compared to normal animals.

Studies of monkeys raised in the wild versus those raised in cages apart from their mothers has indicated that the differences in reproductive behavior between these two groups is less than is generally believed. Both types of animals will accept and care for the infant on vaginal delivery; both groups of males breed normally with evidence for some possible differences in amount of activity, but critical differences occur between the two types of animals in acceptance of offspring delivered by C-section.

In the area of social and reproductive behavior a beginning has been made in assessing normal behavior patterns of the rhesus monkey in the reproductive situation. An understanding is accumulating of the distinctive patterns of behavior of the male and female and of the degree of variation expressed in the caged populations of animals. When proper definition is achieved of these traits of behavior for individual animals, attempts will be made to assess the neural mechanisms underlying and supporting these social and reproductive behavior patterns.

First attempts are underway to explore the neuropathological sequelae of umbilical cord compression during late gestation. It has been possible to reproduce the classical clinical picture of congenital symmetrical spastic diplegia in the rhesus monkey. It is planned to maintain and support the brain damaged infants well into their first or second year of existence in order to characterize the clinical course of their disease and to study the neuropathological changes in the brain under conditions of optimal long-term expression.

A failure of circulation of the fetus has been found in 75 per cent of instances at the time of hysterotomy for umbilical cord compression. This circulatory failure is evident in the poor filling or collapse of the umbilical blood vessels and also in the lack of arterial pulsation in the cord. This results in later abortion of the fetus in 100 per cent of cases. The causes for the circulatory failure of the fetus at the time of hysterotomy has yet to be determined. Compromise of the maternal or of uterine circulation has been posited as one potential cause for secondary alteration in cardiovascular function of the fetus. Support for this possibility has not been found in subsequent experiments. Other hypotheses which may account for the fetal circulatory failure are currently under investigation.

In the area of Biochemistry, earlier work has shown that the dissociation curves of whole blood of the monkey fetus lies 6 to 7 mm. Hg. partial pressure of oxygen to the left of the dissociation curve of adult monkey whole blood at pH 7.4. Subsequent studies of the disposition of the dissociation curves of whole blood in monkey infants of different ages yielded

wide variations from animal to animal with reference to location of the curves. One suspected basis for the wide variability in location of the curves was a possible polymorphism in adult hemoglobin types. However, studies of hemoglobin types among 200 adults in the caged colony using starch gel electrophoresis has given no evidence for polymorphism. On the basis of the absence of hemoglobin polymorphism in the adult and on the basis of comparison of the dissociation curves of different hemoglobin types with those of their respective whole bloods in man it may be concluded that marked variability of the dissociation curves among the infant monkeys may be related rather to an erythrocyte maturation factor which is as yet poorly understood. Other studies have shown that although at the time of birth fetal hemoglobin predominates in the red cells and in whole blood, by the age of about 55 days the proportion of fetal hemoglobin to adult hemoglobin in the blood has decreased to insignificant levels.

A second area of investigation in the area of Biochemistry has been the nature of the transfer or passage of ketone bodies thru the placenta. In vitro studies have shown that NA B-hydroxy butyrate passage through the chorion laevae obeys Fick's law, i.e., penetrates the placenta by passive diffusion. The rate of diffusion of this charged particle thru chorion corresponds closely to that of urea and is slightly more rapid than that for D-arabinose. There was no evidence for active utilization of Na B-hydroxy butyrate by the chorion laevae.

A beginning has been made in vivo studies of the placental handling or transfer of ketone bodies. Rapid injection of Na B-hydroxy butyrate or of sodium acetoacetate in vivo into the maternal femoral vein has revealed that high levels of these compounds can be attained in the blood stream of the maternal animal. Progress of these studies to date have indicated there is rapid interconversion between these two compounds in vivo. Also, although the conversion of Na B-hydroxy butyrate into Na acetoacetate results in levels in the blood to the ratio of approximately ten to one, in the urine the ratio is approximately the reverse. It appears that the excretion of acetoacetate through the kidneys occurs at a much higher rate or at a much lower threshold than that of Na B-hydroxy butyrate. It is hoped further studies will clarify the question of the in vivo transport and permeability of the placenta to ketone bodies.

Several areas of investigation have been explored under the visiting scientists program during the past year. Experimental allergic encephalomyelitis has been produced in six infant monkeys. From six weeks to three months was required for production of clinical symptomatology subsequent to intradermal injection of spinal cord antigen in Freund's adjuvant. There was an acute onset of devastating neurological disease. The animals exhibited varying degrees of hemiparesis or quadriparesis. In two cases the clinieal picture suggested a complete brain stem or spinal cord transection. In addition, there was intention tremor, truncal ataxia, strabismus, anisocoria, unilateral and bilateral facial weakness, opisthotonic posture,myoclonus and seizures. Sensory status examinations were not possible. Four of six animals exhibited retinopathic changes consisting of either a diffuse hemorragic necrosis of the entire retina or of multiple small hemorrages

and areas of exudate. Four of the six animals progressed to a fatal outcome within two to four days while two animals survived into a chronic static neurological picture. Neuropathologically there was restriction of gross findings to brainstem and spinal cord in three animals with additional involvement of diencephalon unilaterally in the fourth. Grossly the lesions appear to consist of micro and macro abscesses and areas of greyish infiltration with necrosis. There were multiple small hemorragic foci in relation to areas of infiltration and gross infarction. Associated was a variable amount of local and generalized brain swelling. There also was evidence of mild meningeal involvement with localized cloudly infiltrates. Microscopically, the lesions in all four animals exhibited a variable picture of acute and chronic inflammatory infiltration of a perivascular distribution with evidence for microhemorrages and foci of diffuse diapedesis of red cells. In one case the polymorphonuclear response was particularly prominent and resembled an acute inflammatory response. In the eyes the lesions were comparable in most respects to the lesions found within the nervous system with perivascular inflammatory infiltrate of both acute and chronic types represented. In many foci, particularly in the retina, the process appeared as a periarteritis with intimal proliferation, thrombus formation and recanalization. In almost all cases there were advanced alterations within the optic nerves. These studies will be extended with variations in the type of antigen and adjuvant used and will be carried out on animals of different ages to more precisely define the clinical variation which this interesting experimental condition expresses in the monkey. (See Project No. NIAID-117, Studies on Auto-immune diseases and related immunologic processes, and NINDB-165 O/CH1212C Ocular pathology and experimental allergic encephalomyelitis in young monkeys.)

During 1964 studies were carried out in the Department of Pharmacology at the University of Puerto Rico School of Medicine through a contractual arrangement. This work was devoted to studies of the effects of chronic denervation on the pharmacological and immunological responsiveness of the diaphragmatic muscle of the guinea pig. It was found that denervated muscle becomes sensitive not only to acetylcholine, but exhibits a marked and unexpected sensitivity to histamine and bradykinin. Furthermore, strips of denervated diaphragm taken from immunized guinea pigs contract in the presence of small concentrations of homologous antigen. Contractions to antigenic proteins appear to have a genuine immunological origin showing all the features of the Schultz-Dale reaction of visceral muscles, namely: 1) They were observed only in diaphragmatic muscles taken from actively or passively immunized animals, 2) They were elicited only by the homologous antigen or structurely related proteins, 3) Repeated antigen administration caused a desensitization and 4) The dose-response curves were bell-shaped showing a maximum peak instead of reaching a final saturational level.

Intracellular electrical recordings of the activity of denervated diaphragmatic muscle fibers during the anaphylactic reaction showed that the shortening elicited by antigen is a true contraction produced by long lasting volleys of action potentials lasting for more than one hundred seconds. Such long-lasting rythmic discharges are not due primarily to depolarization, but rather to an electrical instability of the membrane similar to that produced by decrease in the concentration of calcium ions. It was noted that the surface

membranes of the denervated muscle fibers became highly sensitive to low frequency, low energy, pressure waves in the surrounding solution; a phenomenon which will be further investigated.

These results are of importance because they show that denervated guinea-pig diaphragm develops new receptors to compounds which are inactive before degeneration of the phrenic nerve, findings which offer an opportunity to study the mechanisms by which the motor nerve controls chemical sensitivity of the muscle membrane. The fact that skeletal muscle is capable of showing anaphylactic responses is technically important, also, since striated muscle fibers are much larger than smooth muscle fibers, and, therefore, more suited for the study of electrical events elicited by antigenic action on sensitized tissued.

Studies have continued on the problem of maternal rubella in the monkey and the transmission of infection to the fetus in utero. Studies of fetal tissues, fetal blood, and amniotic fluid has failed to give evidence for the presence of the rubella virus following or associated with rubella infection of the mother. However, serological studies of the blood of the infant has suggested there may be an immunological response on the part of the fetus to the presence of the virus. In the adult monkey, injection of viable rubella virus produces an active immunological response with antibody formation but fails to give evidence of clinical disease. Offspring of monkey mothers who have sustained virus injection during pregnancy have thus far failed to exhibit developmental abnormalities.

Another visiting group has studied arterial perfusion rates of the nervous system of the monkey fetus and newborn. In earlier studies with adult cats it was shown there exists a remarkable differentiation of various neural structures in terms of arterial perfusion rates. There are distinct differences between gray and white matter in perfusion rates. Most interestingly, various regions of subcortical gray matter and various regions of the cortical surface also exhibit wide differences in perfusion rates. Among subcortical structures in the adult, those showing particularly high perfusion rates are the inferior colliculus and the superior olive. Among the areas of cortex, the primary receptive cortex is characterized by rapid perfusion rates compared to other regions of cortex. According to the preliminary results, the rich differential patterns in perfusion rates seen in the adult animal were not seen in the fetus during gestation nor in the infant during the newborn period. Rather, lesser degrees of differentiation were found between gray and white matter on the one hand and no clear differentiation between various regions of the gray matter wherever studied in subcortical nuclei or in the various areas of cortex. (See Project Report M-CS-OC-(C)-10).

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Serial No. NDB(I) 65-LPP 1259

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Neuropathological effects of umbilical cord compression

Principal Investigator: Dr. R. E. Myers

Other Investigator: Dr. W. H. Niemann

Cooperating Units: None

Man Years:

Total:	2.6
Professional:	.8
Other:	1.8

Project Description:

Objectives: To determine the pattern of clinical alteration and neuropathological change secondary to compression of the umbilical cord. To determine the length of umbilical cord compression required for production of neuropathological change at different gestational ages.

Methods Employed: Hysterotomy with exposure of umbilical cord; fetal electrocardiography; compression of the umbilical cord; fetal survival in utero; C-section on the 158th day; assessment of clinical status of infant; extended survival into juvenile life; and finally, histological examination of CNS for pathological change.

Major findings: Seventy-five per cent of the fetuses who have had in utero compression of the umbilical cord have aborted subsequently. The majority of these were noted to have circulatory failure with collapse of the umbilical vessels either at the termination of compression or before its inception. The cause of this intrauterine fetal circulatory failure is not known. Some fetuses sustained up to 20 minutes of cord compression without signs of abnormality on later delivery. Other animals exhibited signs of classical symmetrical spastic diplegia with cord clamp of 20 minutes. The differences between these two results are presumed due to a variable supervention of the above unknown factor which in some animals tends towards circulatory failure in utero.

Significance: These studies, it is hoped, will lead to a better understanding of the etiology of some forms of cerebral palsy. The knowledge of the duration of cord compression which is required to produce signs of CNS damage is of clinical importance. These studies represent first steps in a longer term analysis of the more general problem of the mechanisms underlying conditions for occurrence of, and neuropathological alterations secondary to anoxic brain damage in the fetus and newborn.

Proposed Course: Studies have been exploratory up to this point. It will be necessary to collect data on more pregnancies to gain a picture of the clinical and pathological alterations which may supervene with varying degrees of anoxic insult to the nervous system. In addition, it is most important to pursue studies of the cardiovascular functioning of the fetus in utero under the conditions of C-section in order to explore the identity and reasons for occurrence of the cardiovascular failure seen in up to 75 per cent of the fetuses in the experimental series. It is presumed these changes in fetal circulation may be secondary to changes in cardiovascular function of the mother during surgery or to alterations in blood flow through the uterus. Studies of blood flow and blood pressure in the maternal systemic and uterine circulations would be helpful to test this possibility.

Part B included: No

Serial No. NDB(I) 65-LPP 1260
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: A correlational study of brain lesion effects on a behavioral test battery.

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigator: Dr. Ronald E. Myers

Cooperating Units: Department of Psychology, University of P. R.

Man Years:
Total: .9
Professional: .4
Other: .5

Project Description:

Objective: To develop a method which permits a finer analysis of the nature of particular brain lesions from a behavioral standpoint.

Methods Employed: Individual animal Ss (rhesus monkeys) will be subjected to a number of behavioral tests in automated apparatuses. In each of the tests, stimulus characteristics (e.g. hue, form, number of relevant dimensions, etc.) will be systematically varied.

By examining the performance of the same individual Ss on several different behavioral test situations, a better understanding of the effects of brain lesions, and, conversely, a better understanding of the nature of the behavioral test situations themselves may be attained.

Major Findings: Behavioral situations are currently being designed and constructed.

Significance: Although behavioral tests have long been used to study the effects of brain lesions, progress toward finer understanding of the effects of particular brain lesions, or properties of behavioral tests has been slow and sporadic. The project will investigate effects of cortical lesions on that performance in a more systematic fashion.

Proposed Course of the Project: To complete a workable design of several apparatuses within the next 6 months. The second stage of the project will start thereafter.

Part B included: No.

Serial No. NDB(I) 65-LPP 1261
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
January 1, 1964 through June 30, 1965

Part A

Project Title: The superior colliculus in the goat.

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigator: Dr. Ronald E. Myers

Cooperating Units: Department of Psychology, University of P. R.

Man Years:
Total: .9
Professional: .4
Other: .5

Project Description:

Objectives: To investigate the anatomical projections to and from the superior colliculus in the goat. It is hoped that this will widen our understanding of the functional significance of the structure.

Methods Employed: Nauta-Gygax staining method after production of brain lesion.

Major Findings: The project began in February, 1965. The right eye of two female goats were enucleated and the animals afterwards sacrified. Histological preparations are now complete. This stage of the project has yielded preliminary knowledge concerning proper anesthetic for the goat and experience in histological technique.

Significance: Though in mammals the superior colliculus has declined in its role as the primary way-station of the visual system, evidence indicates it is by no means a vestigial structure. In the goat, the superior colliculus undergoes massive development compared with other species, suggesting a greater functional role in this species.

Evidence suggests the goat may have panoramic vision as compared with central vision in the primates. Further, the oculomotor pattern of the goat differs from that of primates in that pursuing eye movements of moving objects are deficient and there

is no tendency to fixate objects in the center of the visual field.

The study of the superior colliculus in the goat is, thus, expected to give a wider basis for the understanding of its role in the mammalian system.

Proposed Course of the Project: Include studies of efferent projections from superior colliculus.

Part B included: No.

Serial No. NDB(I) 65-LPP 1262

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Reproduction behavior of caged Rhesus monkeys.

Principal Investigators: Mr. Cyril Barnert III
Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.7
Professional:	.2
Other:	.5

Project Description:

Objectives: To study in a detailed and quantitative fashion the reproductive behavior of caged monkeys, with emphasis on the male. Male monkeys are studied to discern individual patterns of behavior and to correlate variations in behavior with such factors as: 1. age and experience of the male, 2. previous period of sex deprivation, 3. compatibility with the particular female, 4. outcome of the mating with respect to insemination and impregnation, and 5. possible seasonal influences on reproductive behavior.

Another eventual objective of the project is to cast light on the neuroanatomical basis of reproductive behavior, as determined by lesion experiments.

Methods Employed: 13 male monkeys from the breeding program are each mated approximately once a week with an estrous female on the 10th through 12th day of her menstrual cycle. The female is brought into the male's cage and left with him for 48 hours. Observations are made by closed-circuit television, from a camera at the cageside, for the first two hours of the pairing. Detailed records are kept of the duration and types of interaction between the two animals, including mounting, ejaculation, grooming, and aggression. Data on the presence of sperm in the vagina after 48 hours, and on the success or failure of conception, are collected.

After characterization of baseline levels, discrete neurological

lesions will be made in a number of the males in areas of the brain which may be involved in instinctive-emotional behavior in order to assess their role in reproductive behavior.

Major Findings: At present the work is in its initial stages. A description of mating behavior for both the male and female in the caged situations has emerged, however, which contrasts with described for the free-ranging monkey, in a number of important respects.

Significance: More immediately, elucidation of the differences between mating in the caged and free-ranging situations may yield an understanding of the basis for the inferior pregnancy yield of the caged monkeys. If the pregnancy rate for the caged colony could be raised to that of the free-ranging animals, there would be a significant increase in the number of pregnancy availability. The major value of this research, however, is in the potential for adding to our at present very meager knowledge of the neuroanatomical, as well as environmental, determiners of reproductive behavior.

Proposed Course of Project: Observations on the mating behavior of the monkeys will be continued in the present format. When sufficient data have accumulated, these will be analyzed in light of the problems already discussed under the objectives of the project. The neurosurgical interventions will be undertaken when it is felt that a sufficiently stable baseline of reproductive performance has been established.

Part B included: No.

Serial No. NDB(CF) 63-LPP 1082
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: In vitro metabolism of ketone bodies by gestational and fetal tissues of the Macaca mulatta.

Principal Investigator: Dr. John J. Schruefer

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.7
Professional:	.3
Other:	1.4

Project Description:

Objectives: 1. To investigate the transport of ketone bodies across the chorion laeve of the Macaca mulatta in vitro.

2. To study the metabolism of ketone bodies in vitro by uterine tissue, by chorion frondosum and laeve, and by various other fetal tissues.

Methods Employed: Chorion laeve studies by the apparatus and method of Battaglia et al., with some improvements and modifications; laboratory synthesis and standardization of β-ketonic acids; pH determinations by glass electrode; colorimetric analysis of ketone bodies; fetal tissue metabolic studies in Dubnoff and/or Warburg apparati.

Major Findings: The study of the in vitro permeability of the chorion, amnion and chorioamnion of the rhesus monkey placenta to Na B-OH Butyrate at or near term is presently approaching completion. Twenty-seven membranes from fifteen pregnant animals have been studied and their permeability constants calculated.

For each of the membranes, 1). The transfer rate of Na β-OH Butyrate is a linear function of the concentration gradient. 2) Over the same range in concentration, the permeability constant is unchanged. Both findings are consistent with Fick's law, governing transfer by simple diffusion. The present studies of Na β-OH Butyrate permeability of the rhesus placenta do not support, therefore, the suggested presence

of carrier systems for certain solute families in the placenta.

The transfer of Na β-OH Butyrate is apparently not altered by adjusting the pH of the tissue media to near the pK of the substrate. Also, it has been previously determined in this laboratory (unpublished data) that a pH gradient will be maintainned by the chorion/chorioamnion within the usual time period of incubation; such a pH gradient apparently does not affect the transfer.

In addition, in preliminary studies it was shown that, for the membranes used and under the usual conditions, there is no significant utilization of the substrate.

Significance: With the chorion laeve as a membrane model, the transfer and utilization of solutes by the specific placental membranes have been carried out in the human, sheep and monkey. In expending our knowledge of the behavior of solute families in this system, it is important to study the free fatty acids and the ketone bodies, to shed some light on what conditions influence whatever exchange of these substances may exist across those membranes.

In addition, an assessment of utilization and/or tolerance of the β-ketonic acids by specific gestational and fetal tissues is important for an understanding of the roles these substrates may plan in vivo, in both normal and pathological states.

Proposed Course of the Project: (1) Over the last year, the number of monkey Caesarean sections has been markedly reduced, and this has affected greatly the course and progress of the project. If sufficient tissue material will be made available, and with the completion of the Na β-OH Butyrate permeability studies, the permeability of the membranes to the related substrate, Na Acetoacetate, will be examined.

(2) The in vitro metabolism of the ketone bodies by rhesus uterine, placental and fetal tissues, in presence or absence of other important respiratory substrates will be studied.

Part B included: No

Serial No. NDB(CF) 63-LPP 1083

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Electrophoretic analysis of cord blood hemoglobins and infant hemoglobins in the Macaca mulatta.

Principal Investigator: Dr. John J. Schruefer

Other Investigators: Dr. André Hellegers
Dr. Frederick C. Battaglia
Dr. M. A. Naughton

Cooperating Units: Department of Gynecology-Obstetrics
The Johns Hopkins Hospital
Department of Pediatrics, University of Colorado, Medical Center
Department of Biophysics, The Johns Hopkins School of Medicine

Man Years:

Total:	2.1
Professional:	.6
Other:	1.5

Project Description:

Objectives: 1. To study by starch gel electrophoresis the adult-fetal hemoglobin patterns in the fetal and infant rhesus monkeys.

2. To study possible correlations between electrophoretically distinguishable patterns and oxygen affinities of fetal and infant whole blood.

Methods Employed: Cord samples drawn at Caesarean section; infant blood samples by venipuncture; hemoglobin preparation by freeze-hemolysis of saline-washed cells; starch gel electrophoresis in continuous (Smithies) and discontinuous (Poulick) systems; assessment of hemoglobin displays by densitometry; conversion to and storage of samples as carbonmonoxyhemoglobin.

Major Findings: Separations of adult and fetal hemoglobin

components of cord bloods and bloods of juvenile animals have been improved by the employment of EDTA-Borate-Tris buffer. Densitometric assessments of the relative amounts of each component have been disappointing so far. The problem presently revolves about the inherent instability of the benzidine staining of the hemoglobin bands and the resultant difficulty in obtaining consistent reliable response calibration of the densitometer. Staining of the hemoglobin displays by other protein stains unfortunately results in shrinkage of the gels and resultant loss of separation of the components. Using the benzidine stain, densitometric measurements have, however, been made with almost the accuracy one can obtain using the Singer, Chernoff and Singer method on human blood.

Significance: A few excellent studies have been carried out previously by others on cord hemoglobin mobilities in the rhesus and other primates, using starch gel and other common media and under various conditions. In the rhesus studies the number of samples examined have generally been small. In addition, quantitation of the relationship of fetal and adult hemoglobins has often been attempted using the Singer, Chernoff and Singer method utilizing the alkali resistance of the fetal hemoglobin.

In earlier investigatons in this laboratory, an attempt to describe the oxygen dissociation curves of the whole bloods of the infant monkey in the transition period from birth to the time when its blood possesses the adult oxygen affinity (already described in this laboratory) was met with the finding of a lack of uniformity in the oxygen affinities of the individual monkey's blood at roughly corresponding ages. The question of possible sources of variation led to the consideration that electrophoretically distinguishable differences in hemoglobins might exist in the infant monkey. So far, however, no real polymorphism has been found. The study continues, however, as a quantitative assessment of fetal/adult hemoglobin proportions in the fetus and maturing infant, as determined by electrophoretic separation and densitometry.

With the findings of ours and others that in the cord blood samples of the rhesus, fetal hemoglobin is quite consistently at a high level, in contrast to the greatly varying proportions of adult and fetal hemoglobin in human cord samples, it is perhaps not as easily said in the rhesus, as it is on the human, that superimposability of oxygen affinities at identical pH is strong evidence that the difference between the whole blood O_2 affinities of adult and fetal monkeys cannot be due to the amount of alkali-resistant hemoglobin present. Furthermore, if such does turn out to be the case, perhaps the transition oxygen affinity curves will be able to be correlated with fetal/adult hemoglobin proportions.

Proposed course of the Project: (1) Presently studies to

improve the stability of the benzidine stained bands are being carried out, in addition to the search for another suitable indicator. (2) With these techniques further refined, we will return to the study of the "transition O_2 curves" to look for possible correlation. (3) Fetal hemoglobin samples as separated by electrophoresis will be collected and examined by a cooperating unit for polypeptide and amino acid sequence studies in the future.

Part B included: No

Serial No. NDB(CF) 63-LPP 1080
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Experimental ketosis in the pregnant rhesus monkey.

Principal Investigator: Dr. John J. Schruefer

Other Investigators: Dr. Wendell H. Niemann

Cooperating Units: None planned.

Man Years:

Total:	1.9
Professional:	.4
Other:	1.5

Project Description:

Objectives: 1. To investigate the transplacental transport of ketone bodies in the pregnant Macaca mulatta.

2. To study the possible effects of exogenous maternal ketosis on fetal existence and welfare.

Methods Employed: Laboratory synthesis of ketone body; manometric standardization of infused β-ketonic acid; infusion of ketone bodies into maternal circulation; determinations of uterine A-V differences, fetal blood levels and amniotic fluid levels of ketone bodies; pH determinations via glass electrode; starch gel electrophoresis for maternal serum, fetal serum and amniotic fluid protein pattern changes; enzymatic blood glucose determinations.

Major Findings: This study has been delayed by the lack of assigned pregnant animals. However, several preliminary experiments have been carried out using non-breeding females to see how they handle intravenous infusions of relatively large amounts of Naβ-OH Butyrate and Na Acetoacetate. More specifically, the purpose of these studies was to determine: 1. What levels of these substrate could be attained in their systemic circulations. 2. What effects, idiosyncratic or otherwise, might result. 3. How fast the animals could be expected to clear the substrate. Briefly, it has been

found that: 1. Appreciable arterial and venous levels can be reached and maintained in the face of rapid renal excretion by by continuous infusion of the salts. 2. Interconversion of Naβ-OH Butyrate and Na Acetoacetate occurs rapidly. 3. A pronounced hypoglycemia occurs immediately and continues with infusion. 4. Little systemic pH change occurs with the infusion of the neutral unbuffered salt solution. 5. No apparent idiosyncratic reaction occurs and all four animals have survived large doses of either substrate without difficulty.

Significance: The β-ketonic acids found in normal animals and at elevated levels in diabetes and starvation are known to be respiratory substrates, formed as side reactions to fatty acid degradation. To add to our present knowledge of transplacental exchange of gasses and other nutriments, the behavior of these substrates in the maternal-placental-fetal relationship will be studied.

Proposed Course of the Project: 1) To establish an exogenous maternal ketosis in the Macaca mulatta, to study the transplacental handling of the particular acids chosen by chemical analysis of fetal tissues. 2) Should appreciable levels of these substrates be then found in the fetal circulation, a critical examination of fetal welfare from the standpoint of other parameters will be necessary.

Part B included: No

Serial No. NDB(CF) 63-LPP-1081
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Electrophoretic analysis of the hemoglobins of a large colony of Macaca Mulatta.

Principal Investigator: Dr. John J. Schruefer

Other Investigators: Dr. M. A. Naughton
Dr. F. C. Battaglia
Dr. A. E. Hellegers

Cooperating Units: Department of Biophysics, The Johs Hopkins School of Medicine
Department of Pediatrics, University of Colorado Medical Center
Department of Gynecology and Obstetrics of the Johns Hopkins Hospital

Man Years:

Total:	2.2
Professional:	.7
Other:	1.5

Project Description:

Objectives: 1. To analyze by starch-gel electrophoresis the hemoglobins of the adult members of a large colony of rhesus monkeys in a search for abnormal hemoglobins.

Methods Employed: Venipuncture; hemoglobins obtained from quick-freeze hemolysates of saline washed cells; conversion to and storage as carbonmonoxyhemoglobin; standardization of concentration by hand protein refractometer; starch-gel electrophoresis in cold by Poulick or Smithies system.

Major Findings: 1. Approximately 200 animals of the San Juan colony and 15 animals from Cayo Santiago and La Parguera have been examined so far and no polymorphism has been found as yet. 2. No minor hemoglobin components have been found in those animals subjected to examination. 3. The major (A) component of human adult samples run in our laboratory has a mobility identical to that of the adult rhesus hemoglobin, as has been the experience of others. The human A_2 component has been demonstrated nicely.

Significance: A review of the literature reveals that so far a search for biochemical polymorphism of hemoglobins in the rhesus monkey has been unrewarding, although abnormal hemoglobins have been found in other non-human primates. This continues to be surprising to those interested in the biochemical genetics of the hemoglobins and the feeling persists that perhaps not enough animals have been examined. In addition, no minor hemoglobin components have been found in these animals, leading to the suggestions that perhaps the genetic structural locus controlling δ-chain synthesis arose relatively recently on the evolutionary timeable as a duplication of the locus controlling the more ancient β-chain. In spite of the fact that much evidence supports this hypothesis, the search for the A_2 or other minor hemoglobin components in the rhesus continues. Our large colony of Macaca mulatta appears to be an excellent source for this kind of information and has not been tapped previously for these data.

Proposed Course of the Project: (1) To examine the hemoglobin pattern of every monkey in the primate caged breeding colony and perhaps to extend the survey to the animals in the field colonies of free ranging monkeys. (2) In the case that an abnormal hemoglobin is found, a) the hemoglobin will be studied by a collaborating unit for polypeptide arrangement, amino acid sequence and other important molecular characteristics; b) genetic experiments may be carried out with the animal involved; c) the hemoglobin will be studied in this laboratory and by collaborating units as to its oxygen affinity, Bohr effect, whole blood oxygen affinity and other important physiological and biochemical characteristics.

Part B included: No

Serial No. NDB(CF) 61-LPP 820

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Effects of controlled early experience on development of the Siamese Kitten.

Principal Investigator: Dr. Gilbert W. Meier

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	.1
Other:	1.1

Project Description:

Objectives: To explore the parameters of controlled infantile stimulation which lead to behavioral and physical growth precocity.

Methods Employed: Earlier researches by this investigator have shown that increasing the amount and nature of early experience, as through handling, produces a number of changes which, together, indicate precocious development in the stimulated subject. The range of effective stimuli which have this provocative effect is being studied presently. (1) In the first experiment, litters of kittens were stimulated by moderate light and tones, 15 min. daily for the first 30 days after birth while recordings were made of general activity and vocalizations. Litter-mate controls were placed in the same apparatus without the additional stimulation but with the appropriate recording procedure. (2) In a second experiment, mild electroshock (0.3 ma.) was the stimulus given the experimental kittens during the first 30 days. Control and recording procedures were those used in (1).

Major Findings: The striking differences in the development of coloration patterns noted in the handling studies were noted when the stimulation was light and sound. In every litter studied, the

lightest animal at 30 days (the age when the differences are most marked) was an unstimulated subject; the darkest, a stimulated subject. In two of the five litters, all stimulated kittens were darker than the controls. All coloration differences appeared with concomitant indications of differences in behavioral activity and reactivity during the stimulation procedures. The data collected to date on conditioning and emotionality at four months of age indicate that the stimulated kittens are more responsive to the testing situation.

Significance: These studies indicate conclusively that the enhancing effect of the early experience of handling has little to do with the nature of the stimulation, per se, certainly nothing to do with a mystical or psychoanalytic connotation of handling. Precocious development can be induced by any reasonable (within reasoned extremes) form of peripheral stimulation.

When the concept of early experience is proffered, a diversity of conditions is immediately implied but only vaguely specified. Studies such as these serve to make more concrete our notions of effective, and ineffective conditions, and of the developmental ages when they can be appropriately applied.

Proposed Course of the Project: Project will be concluded with the publication of the results of the studies.

Part B included: Yes

Serial No. NDB(CF) 61-LPP 820

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Meier, G. W.: In Search of the Engram: In the Nursery. In G. Newton (Ed.), Early Experience and Behavior. Springfield, Ill., C. C. Thomas (in press).

Serial No. NDB(CF) 61-LPP 821
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Early development of emotional learning in monkeys

Principal Investigator: Dr. Gilbert W. Meier

Other Investigators: None

Cooperating Units: None

Man Years:

Total	1.7
Professional:	.2
Other:	1.5

Project Description:

Objectives: This series of studies proposes to explore carefully the development of learned emotional behavior during early infancy in the monkey (Macaca-mulatta) by behavioral and electrophysiological means.

Methods Employed: Groups of infant monkeys (n = 4) are being tested for the development of conditioned emotional responding in the classical and operant conditioning paradigms. The desired response is of two types: 1) the Conditioned Emotional Response (CER): an alteration in activity (decrease or increase in activity level) with the onset of the conditioned stimulus (3000 cps. tone at 75 db.); 2) the Conditioned Avoidance Response (CAR) (in the operant procedure only): the vocalization during the presentation of the conditioned stimulus prior to the onset of the unconditioned stimulus (electroshock: 300 VAC at 1.0 ma.).

In the classical conditioning paradigm, the subjects, vaginally-delivered, are tested in 5-day sessions at 0, 30, 60, and 90 days of age (Group I: CC) or at 30, 60, and 90 days of age (Group II: CC). In the operant conditioning paradigm the subjects, vaginally-delivered, are tested in 5-day sessions at 0, 30, 60, and 90 days of age (Group I: OC-VD); at 30, 60, and 90 days (Group II: OC-V/D), or at 60 and 90 days (Group III: OC-V/D). A last group (Group IV: OC-V/D) is tested in 5-day sessions at 15 and 30 days of age, only.

Two additional groups of subjects, delivered by cesarean section, are being tested under the operant conditioning paradigm and will be comparable to the Groups I and II which were vaginally-delivered. (Groups I: OC-C/S and II: OC-C/S).

In other subjects, EEG and EKG recordings have been made during the process of classical emotional conditioning. The subjects, unrestricted save by the recording leads, were tested repeatedly at two-week intervals starting at either the second (n = 2) or tenth (n = 2) day of life and continuing until the ninety-fifth day.

Major Findings:

1) Classical conditioning paradigm: The learning of the CER is rapid, in terms of both age and number of trials, showing itself within the first twenty-five trials of the first testing session. Some learning of this variety is evident in the records of the youngest subjects, i.e., within the first five days of birth. After repeated testing most subjects show a depression of activity and reactivity.

2) Operant Conditioning paradigm: The appearance of the CER is as early in these groups of subjects as those tested under the classical paradigm. However, the appearance of the CAR is much slower with little substantial evidence of its existence in the earliest records of the youngest subjects but significantly so in later records. The initial effect of the conditioned stimulus in the youngest animals (Group I: OC-V/D) is an increase in the level of activity and in the frequency of vocalizations without the previous pairing of the auditory stimulus with the electroshock. This phenomenon is not so apparent in those subjects first tested at a later age (Groups II, III, and IV). With repeated testings at the earliest ages, the animal adapts and later comes to respond in a truly learned fashion. Overall, the performancesof these animals indicate a significant effect of experience in the experimental situation but no effect which can be attributable to maturation or experience in the nursery, that is, there is no phenomenon which can be related to the maturational age, critical period, or to early experience. Essentially, the performances of the animals at 30 days of age and beyond, can be described by a series of parallel curves related entirely to number of previous trials.

Those infants born by cesarean section responded very differently in the operant paradigm as compared to the vaginally-born animals. In general, their levels of activity, frequency of vocalization and level of reactivity was diminished, especially so in the group of animals tested in the first five days after birth (Group I: OC-C/S). This effect was not so striking when first tested on the thirteeth day. In the latter group (Group II) OC-C/S), the animals demonstrated a unique interaction, in terms of activity and vocalization levels, with repeated

testing within a given session not shown by their vaginally-born counterpart.

No electrophysiological correlates could be found indicative of the process of learning the CER in the classical conditioning paradigm. Although these groups of subjects demonstrated the establishment of a conditioned response, the only discernible electrophysiological pattern was one indicative of an overall, long-term process of adaptation, resembling those patterns otherwise seen in certain phases of sleep (the slow-wave phase). The general activity records confirmed this state of sleep or mild inhibition.

Significance: Since current psychological and psychiatric thought places heavy emphasis on the role of early experience in development of emotional behavior, detailed understanding is necessary of what the infant is responsive to and the manner in which he comes to respond emotionally.

Proposed Course of Project: This project will be completed within the first months of the next fiscal year. At that time the data will be analyzed and prepared for publication.

Part B included: No.

Serial No. NDB(CF) 61-LPP 822
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The effects of infantile trauma and social isolation during rearing upon adult reproductive behavior in the rhesus monkey Macaca mulatta.

Principal Investigator: Dr. Gilbert W. Meier

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.5
Professional: .2
Other: 1.3

Project Description:

Objectives: Recent reports from the Wisconsin Laboratory have described the devastating effects of pre-adult social deprivation upon the reproductive behavior of rhesus monkeys. The present project is: 1) a replication of those reports under comparable conditions; and 2) an investigation of the effects of the early experience variables in conjunction with mode of delivery, i.e., vaginal vs. surgical delivery.

Methods Employed: 1) Observations were made on six male and six female rhesus monkeys, 3-5 years of age. All animals were born and reared in this laboratory. Two of the males and four of the females were asphyxiated at birth. All infants were reared in social isolation, receiving only that human contact necessary for the collection of routine physiological data and maintenance of health. Upon reaching sexual maturity, the monkeys were incorporated into the regular breeding colony. The monkeys only regular contact with other animals occurred during the course of the 48-hr. breeding sessions.

After 157 days of gestation, the female was removed to special

facilities so that the parturition could be observed as well as the initial contacts between the female and her infant.

2) Observations on the acceptance of the infant and reaction to human intervention were made of thirteen female monkeys, six laboratory-reared and seven feral-reared, following the surgical delivery of their infants. After the surgical delivery of the infant, and the settling of the female in her home cage, the experimenter introduced the infant to the female. He observed, in the subsequent two hours and for two-hour intervals on the two succeeding days, the reaction of the female to her infant and her reaction to the experimenter.

Major Findings: 1) Ten of the 12 animals demonstrated reproductive behaviors with a frequency and at an age comparable to wild-born and raised monkeys. In the group of 6 females, 8 pregnancies have been recorded. Three were terminated surgically; 2 for purposes other than those of this study. The 4 females (5 pregnancies) who delivered vaginally, approached, cleaned, and clasped the newly-born infant at the normal time and in the normal manner. Two of the females were permitted to care for their infants during the subsequent three months. This they performed adequately.

No significant differences in these reproductive behaviors could be discerned between those monkeys, male and female alike, which had been asphyxiated at birth and those which were the unasphyxiated controls.

2) None of the laboratory-reared females responded appropriately to her surgically-delivered offspring in any of the three test sessions. In contrast, all of the feral-reared females responded appropriately by the second day; one female took the infant from the experimenter's hands on the first presentation and immediately clutched it to herself; the others responded appropriately either later in the first session or sometime during the second. Further, the laboratory-reared females showed none of the aggressive or threatening behaviors to the experimenter typical of the female monkey with young whereas all of the feral-reared females responded vigorously but with somewhat greater variability in the intensity of response than was shown for the acceptance behaviors.

Although maturational and experiential differences existed between the two groups of females they do not appear to be related to the observed differences in maternal behaviors.

Significance: The contrast between the data from these studies and those reported from the Wisconsin laboratory is overwhelming. No significant differences in the two laboratories regarding the care of the young monkey have been recognized which could account for the discrepancy in the observations. Those differences which do exist

indicate that emphasis placed on physical contact and play is exaggerated.

Reasonably, for the elicitation of adequate maternal behaviors in any adult female monkey a number of conditions must be met, collectively and in interaction. Those conditions may be either ontogenetic or contemporary but no one of which, however, is either necessary or sufficient. The unique conditions which lead to the acceptance in one experiment (response following vaginal-delivery) but to the rejection in the other experiment (response following surgical-delivery) and which could explain the differences in the behaviors of the laboratory- and feral-reared females are: (1) a fundamental difference in the endocrinal systems of the two groups of females as dependent upon the rearing experience; (2) the absence of the endocrinal changes and the stimulus complex which attend natural parturition, in both laboratory- and feral-reared groups; and (3) the presence of an infant whose vocal responding is less than normal, in both groups. Each of these, in conjunction with, but not independent of, the ontogenetic uniquenesses of the animals could make the present observations understandable. The likelihood of the development and evocation of adequate maternal behaviors is over-determined such that only when several of these conditions are lacking, e.g., established social behaviors, current appropriate endocrinal state, and effective infantile stimulation, will effective maternal-infant relations not be established.

Proposed Course of the Project: This project will be terminated this fiscal year (FY 1965) with the publication of the results of the two major studies.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Meier, G. W.: Other data on the effects of social isolation during rearing upon adult reproductive behavior in the rhesus monkey (Macaca mulatta). Animal Behav.(in press).

Meier, G. W.: Maternal behaviour of feral- and laboratory-reared monkeys following the surgical delivery of their infants. Nature (in press).

Serial No. NDB(CF) 63-LPP 1075
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The effects of neonatal damage to the inferior colliculus upon the physiological and behavioral development of the rhesus monkey (Macaca mulatta).

Principal Investigators: Dr. Gilbert W. Meier
Dr. Donald P. Foshee

Other Investigators: Dr. Orlando Andy

Cooperating Units: Department of Neurosurgery
University of Mississippi Medical Center
Jackson, Mississippi
Department of Anatomy, School of Medicine
University of Puerto Rico
San Juan, Puerto Rico

Man Years:
Total: 1.6
Professional: .2
Other: 1.4

Project Description:

Objectives: Since histopathology of the inferior colliculus is one of the most common sequelae of experimentally-produced neonatal asphyxia, an understanding of the significance of lesions in that structure without the physiological complications of asphyxiation is necessary for the interpretation of the behavioral deficits of that form of neonatal trauma.

Methods Employed: Lesions have been placed, bilaterally, in the inferior colliculi of six infant monkeys, vaginally-delivered, and six infants, surgically-delivered. All lesions were placed stereotaxically with coordinates established on a group of infants from the laboratory museum and from four infants sacrificed within three months of the operation. All lesions were made under local anesthesia within twenty-four hours of birth. A sham-operation was performed on an additional three vaginally-delivered infants and two surgically-delivered infants which serve as controls.

Initially, recordings were made from cortical (scalp) electrodes and from the electrodes in the inferior colliculus under conditions of quiescence and of auditory or visual stimulation. Recordings were also made from the scalp electrodes following direction stimulation to the inferior colliculus. However, since no unique or characteristic response could be recorded under either condition whereby the anatomical positioning could be confirmed physiologically, the practice was discontinued.

Two of the vaginally-delivered, experimental animals were prepared for the recording of electrophysiological and behavioral parameters of sleep and wakefulness during the first month after birth and of behavioral parameters alone during the two months following. The other animals, controls and experimentals, were tested under the operant conditioning paradigm (see report, Serial No. NDB(CF-61-LPP 821) when 0, 30, 60, and 90 days of age. At each age the testing was repeated for five consecutive days. After these recordings, when about 95-100 days of age, all animals were shipped to the Laboratory of Experimental Behavior, University of Mississippi, where the behavioral testing and the electrophysiological recording continue. There activity in a free field, activity to novel stimulation, discrimination performance, aspects of perceptual development, and, ultimately, social responding are being recorded.

Major Findings: Marked motor impairment has been observed in the majority of the experimental infants observed. This impairment consists of abnormal positioning of the head and limbs, in sluggish righting responses, in poor grasping responses, and in a tendency toward seizure-like patterns. In contrast with other infants seen in this laboratory with seizure behaviors, these infants remain unusually quiet with minimal vocalization. Although most evident during the first days of weeks following birth and the operation, vestiges of these motor impairments persist until at least eight months of age. In spite of their motor difficulties, all infants have been able to feed and suck adequately although, possibly, somewhat belated in their establishment of a self-feeding regimen (10-15 days as against 5-10 days).

No motor or maintenance difficulties have been observed in the sham-operated controls.

The sleep and wakefulness patterns of the operated infants are essentially normal (see report, Serial No. NDB(CF-63-LPP 1077) except for certain inversions during the first 5 days of recording. The proportion of time spent asleep and the proportion of that time spent in paradoxical or rapid-eye movement sleep seems high but the data are insufficient to determine whether it is significantly so. The patterns

and cycles of activity and inactivity are also within the normal or expected range.

The most striking feature of the conditioning data is the absence in all animals of the typical responding to the conditioned stimulus during the first days of the first session and the absence of any subsequent learning of the avoidance response to this stimulus. Between the groups however, marked differences in level of activity and frequency of vocalization were observed as related to mode of delivery and experimental treatment. Overall, the surgically-delivered controls tended to be the least active and the least vocal whereas the surgically-delivered operates were the most active and the most vocal.

Later, when about six months of age, the monkeys have shown differences in exploratory activity which indicate a lessened emotionality and/or a heightened exploratory tendency among the operated subjects.

Significance: In the understanding of the long-term effects of infantile brain damage, particularly that induced by neonatal asphyxia, considerable confusion exists regarding the relation between the anatomical changes, acknowledged or not acknowledged, and the behavioral changes, real or imagined. Only recently have the loci of neural damage clearly attributable to the asphyxiation, per se, been reliably described. By contrast with the older, clinical literature, the experimentally verified damage is restricted to the brain stem, particularly to the major sensory pathways, most prominently to the ventral thalamus and the inferior colliculus. This information, however, does not aid the understanding of the intellectual and emotional alteration which may follow this form of infantile trauma since such centers are not recognized for their involvement in these behaviors (if the lesions are produced in adulthood) and since sensory deficits appropriate to these centers have never been shown. A reasonable explanatory principle for this peculiar state of affairs, other than epiphenomenalism, is that the brainstem damage makes the infant less active and less reactive to his environment and, thus, effectively duplicates the outcome of an intact infant reared in an impoverished or non-stimulating situation. Some of the data collected on asphyxiated infants studied in this laboratory tend to confirm this proposition.

Proposed Course of the Project: This project will be terminated with the conclusion of the current fiscal year (Fy1965). The animals have been sent to the Laboratory of Experimental Behavior, University of Mississippi, for continuing study there. Collaboration will continue between the principal investigators who will prepare reports of the project as the data are accumulated and analyzed.

Part B included: No

Serial No. NDB(CF) 63-LPP 1076
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Infantile Trauma: The role of genetic and environmental factors.

Principal Investigator: Dr. Gilbert W. Meier

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.8
Professional: .1
Other: 1.7

Project Description:

Objectives: To evaluate the contribution of genetic and experiential factors in the expression of long-term effects of infantile trauma in the context of the persisting behavioral differences which can be attributed to the genetic differences in the parental population.

Methods Employed:

(1) The many conflicting reports regarding the significance of infantile trauma to the development of behavior implicate genetic and environmental factors exerting an attenuating influence. As a first check on this possibility, infant mice of two strains and their hybrids were deprived of oxygen (nitrogen asphyxiation) for 45 minutes within 12 hours of birth. Some of the subjects (n= 5 for each dimension of each variable: treatment, sex, and genetic origin) were tested for activity and reactivity when 14 days of age; others (n= 10 for each dimension of each variable) were similarly tested when 63 days of age.

These measures consisted of counts from an ultrasonic activity recorder generated by the subject's movements under one of three standardized conditions: 15 min. in dark; 15 min. in light; and 15 min. in flashing light and pulsing tone. The younger animals were tested but once under these conditions; the older, three times,

on three successive days.

When 105 days of age, a third group (n= 10 for each dimension of each variable) was trained on a water maze for the acquisition of a position habit and its subsequent reversal.

(2) Groups of mice (n= 5) representing both sexes and both parental strains, C57BL/6 and BALB/c, are being tested at 3, 6, 9, 12, 15, 21, 27, 33, 39, 45, or 51 weeks of age on the solution and recall of a water-maze problem. Four days, five trials per day, are given to the learning of the original problem and two days are given to the learning of the reversal of the original solution.

Major Findings: Although most of the data have been gathered, the processing is still far from complete. However, present indications are that the orienting hypothesis is tenable, that is, that certain genetic constellations make the subject more susceptible to neonatal trauma and that these may be factors related either to homozygosity or to unique homozygous variants.

Evident in all of the data, is the dominant effect of strain difference. By and large, the BALB/c mice react minimally to changes in the ambient environment and are poor performers in the water maze. In general these characteristics increase with age but diminish late in life. In contrast, the C57BL/6 animals respond readily and perform well at all ages although their performance, too, deteriorates with age.

Significance: In the researches on the topic of mental retardation, the overwhelming portion of energies is given to the discovery of causes and the specification of conditions. The type of research undertaken here is in the hope of bridging the gap between one facet of behavioral theory, the effects of early experience, and one of social demand, the treatment of mental defectives. Since the long-term effects of neonatal asphyxiation are fairly well known and their contribution to behavioral dysfunction well agreed upon, this form of infantile trauma is an obvious choice. The demonstration of an amelioration of the asphyxiation effects will be all the more convincing for a positive approach to the education and care of retardates.

Proposed Course of the Project: With the completion of the last groups of mice on the water maze and the publication of the results, this project will be terminated. Expected date for completion: 1 September 1965.

Part B included: Yes

Publications:

Foshee, D. P., Vierck, C. J., Jr., Meier, G. W., and Federspiel, C.: Simultaneous measure of general activity and exploratory behavior. Percept. mot. Skills, 20: 445-451, 1965.

Meier, G. W.: Differences in maze performance as a function of age and strain of housemice. J.C.P.P., 3: 418-422, 1964.

Meier, G. W., and Foshee, D. P.: Albinism and water escape performance in mice. Science, 147: 307-308, 1965.

Serial No. NDB(CF) 63-LPP 1077
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Studies on the development of sleep and wakefulness patterns in the Macaca mulatta.

Principal Investigators: Dr.Gilbert W. Meier and Dr. Ralph J. Berger

Other Investigators: None

Cooperating Units: Department of Psychology
University of Puerto Rico
Rio Piedras, Puerto Rico

Man Years:
Total: 2.7
Professional: .8
Other: 1.9

Project Description:

Objectives: These studies on sleep and wakefulness activities are designed, first, to explore the development of sleep-wakefulness patterns in the infant and juvenile rhesus macaque; second, to delineate some of the more obvious environmental and experiential parameters in their development; and third, to evaluate the possibilities of modification of these patterns through the manipulation of the infant per se or the environment in which he must be reared.

Methods Employed:

Group A: Five infant monkeys, four delivered vaginally and one surgically, were reared on a mother surrogate under continuous light conditions in a sound-shielded environment (attenuation: about 70db for frequencies above 100 cps.) until one month of age. Superficial electrodes were placed on the head for the recording of the EEG (two channels) and EOG, on the neck for the recording of EMG, and on the chest for the recording of the EKG and of respiration. These electrodes were sealed with collodion and offered minimal disturbance to vital functions. The surrogate situation provided for adequate maintenance and thereby minimized experimenter intervention with the recording process. Twenty-four hour records were made for the first

seven days of life and on alternate days thereafter for the balance of the month.

In addition, continuous recordings were made of the overall activity (ultrasonic activity recorder), vocalizations, and sucking of these animals. These recordings continued on three of the infants until they were three months of age.

Similar electrophysiological recordings were made for a 72-hr. period (minimum) on three juvenile monkeys (9-13 months of age) which had been reared in the colony. Electrode number and placement were comparable to those used with the infants. Rather than a surrogate situation, these animals were maintained in a primate chair and hand-fed at regular intervals. No measures of gross activity or vocalizations were recorded from these subjects.

Two additional full-term, vaginally-born infants are being reared on a mother surrogate but in an environment which has marked changes in ambient stimulation (general laboratory environment). Recordings are made of general activity, external auditory stimulation, and of vocalizations.

Group B: Five vaginally-delivered infants are being reared on mother surrogates in a normal environment but without patterned vision. (The latter is accomplished by the insertion of translucent occluders under the lids over the cornea.) Recordings (EEG, EOG, EMG, and EKG) are made at two-week intervals beginning with the day of birth. Five additional infants are serving as the full-visioned controls.

The electrophysiological recordings and the unique rearing conditions will continue until significant changes occur in particular aspects of certain sleep states (diminution of the rapid eye movements in the paradoxical or low-voltage, fast wave phase of sleep without similar diminution during wakefulness) or until one year lapses, whichever is first. At this time, the occluders will be removed and the animals will be tested for the typical signs of perceptual deprivation and the eventual (?) assumption of normal behavior as well as for the recovery (assuming that a diminution has occurred) in the electrophysiological signs of paradoxical sleep.

The observations from these animals in conjunction with comparable observations from their controls should furnish information as to whether the rapid eye movements of paradoxical sleep represent movements associated with scanning or searching actions and on the process of perceptual deprivation which eventuates in the typical signs of this state as reported by other investigators.

Group C: Two groups (n = 3) of vaginally-delivered infants and two groups (n = 3) of juveniles (9-10 months) have been selectively deprived of either the slow-wave phase of sleep or the paradoxical (low-voltage, fast wave) phase of sleep. The deprivation was accomplished by the automatic presentation of a combination of a 3000 cps. tone (up to 100 db.) with electric shock (up to 8.0 ma.) at the onset of the particular sleep phase and subsequently increasing the intensities of both until a change to the other state of sleep or wakefulness ensued. Deprivation of either state continued until the presentation of stimuli was no longer effective or until the lapse of eight days of deprivation, whichever was the earlier. Thereafter the animal was permitted a recovery period, during which the several electrophysiological measures (EEG, EOG, EKG, and respiration) were also recorded, and which lasted as long as the deprivation period.

These observations should aid in the establishment (or rejection) of the concept of "physiological need" of these sleep states as well as lend further credence to the similarity of the infantile and juvenile states.

Group D: Seven premature infants (two vaginally-born, one at 137 days, one at 144 days; surgically-born, two at 136 days, two at 140 days, and one at 146 days) have been prepared and arranged for the continuous recording of the behavioral and electrophysiological indicants of states of sleep and wakefulness (see Group A, above). Successful recordings had been made on the one vaginally-born, 137-day infant for two weeks when the procedure was discontinued for reasons other than this experiment, and on the two born at 144-146 days for one full week about which time the infants expired, probably through aspiration of fluid. The other prematures died within 24 hours of delivery even though special precautions were taken because of the immaturity of the subjects (frequent hand feedings, rearing in cloth-lined box rather than on surrogate, etc.).

Group E: Three full-term, vaginally-born infants will be prepared as were the infants in Groups A and B (with electrode on tail for electroshock) for the recording of the electrophysiological indicants of sleep and wakefulness and for the presentation of stimulation (electroshock to 4.0 ma.) during selected periods of each of the three states (wakefulness; high-voltage, slow-wave sleep; low-voltage, fast wave sleep). Thus during each 24-hr. period, throughout the first four weeks of life, the infants will be presented automatically with electroshock whose level will increase until full wakefulness (vocalization) is reached during ten periods of each of the three states. In this way the threshold to noxious stimulation (the level of arousal or the depth of sleep) will be determined as related to the particular state and to the developmental or maturational age.

Major Findings:

Group A: The three states of vigilance: wakefulness; high-voltage, slow wave sleep (HVS); and low-voltage, fast wave sleep (LVF) exist in the same form as characterized by the established polygraphic and behavioral patterns of the adult, although in varying temporal relations. For example, the rise from birth in the total sleep time and that proportion of it occupied by LVF persists until the seventh day of life. Thereafter, the total sleep time remains constant while the proportion of LVF declines into juvenile life. In addition, the mean durations of periods of wakefulness, sleep, HVS, and LVF, show marked changes on this day. The uniqueness of the seventh day of postnatal life for the development of the infant monkey is further emphasized by the initial appearance at this age of a number of important behavioral responses.

No differences were revealed in the polygraphic or behavioral patterns which could be attributed to mode of delivery.

As the infant matures, the periods of sleep and wakefulness become "consolidated", that is, not only does their mean duration increase but the particular phase of the 24-hr. day in which they appear becomes more predictable. In other words, the subjects develop sleep-wakefulness cycles. Those animals reared in the normal laboratory environment have cycles related to changes in levels of ambient stimulation; those reared in the light-constant, sound-shielded environments have cycles independent of the laboratory stimulation with, possibly, something other than a 24-hr. period (probably a longer period). The establishment of these cycles appears to require six to ten weeks.

Group B: Some changes in the developmental pattern of the EOG during LVF is evident in the experimental animals after seven to nine months in the experimental conditions. One infant (now juvenile) exhibits a marked reduction in the frequency, amplitude, and velocity of its eye movements during this phase of sleep (LVF). Two other experimental animals show some reduction in these movements but to a lesser degree. The sighted animals show a slight reduction in the amplitude of the eye movements during LVF, but without alteration in frequency or velocity. No such diminution in the eye movement parameters is evident during the period of wakefulness in any of the monkeys. However, all of the experimental animals exhibit marked and chronic nystagmus during wakefulness by the time they reach six months of age (evident in closely observed animals before the end of the first month).

All animals have developed normally under the rearing and experimental conditions. The deprived animals, like the normal animals, are vigorous and active. When in their home cages their plight can only be surmised by careful and knowing observation.

Group C: The analysis of the data completed to date indicates that both the neonates and the juveniles display a progressive increase in threshold to the tone-shock combination during the deprivation of either phase of sleep. However, the neonates increase their thresholds at a faster rate and reach a level of 8.0 ma. of electric shock around the seventh day of deprivation, whereas the juveniles reached a threshold of only 5.0 ma. following a comparable period of deprivation.

Post-deprivation or recovery effects of either state are not evident in the records of the neonates when normative, developmental data are considered. On the other hand, the juveniles subjected to the deprivation of LVF demonstrated significant compensatory recovery effects specific to that state of sleep following the termination of deprivation. The juveniles subjected to the deprivation of HVS demonstrate some specific recovery effects but to a lesser degree.

Group D: The only successful maintenance of the premature infants has been with those born close to full term or those born vaginally. This is consistent with other experiences in this colony and may implicate significant physiological or endocrinal differences in the infants as related to mode of delivery.

The patterns of sleep and wakefulness, polygraphically defined, are difficult to discern during the first 24 hours, or so, in those infants born after only 136 days of gestation. Reliable patterns, relatively easy to discern, do emerge in the period immediately following. The temporal patterns of the states of sleep and wakefulness in these prematures are somewhat different from those born at full-term. The time spent in sleep and the proportion of that time spent in LVF are at their maxima during the first 24-72 hours and decrease continuously through the remainder of the recording periods. The proportions of the two, of sleep and of LVF, are not as high as would be expected from a belief that these states are more "primitive" and indicate a simpler and more basic functioning of the individual.

Group E. This group is just starting and have given no analyzable data as yet.

Significance: Developmental studies in kittens and in human infants have shown sleep with rapid eye movements to occupy approximately 50% of total sleep time at birth gradually diminishing to 20 - 25% in the adult. However, important differences in the EEG accompanying the varying states at birth exist between species. Differential EEG patterns accompanying wakefulness and sleep states are present in the human neonate but only develop completely at 5-6 weeks of age in the kitten.

Beyond the obvious value of exploring the problems of activity stages and cycles in a species other than those previously mentioned,

in a research setting, and with a research subject which would permit optimal experimental control and manipulation, the results of this series of studies directed to the description and understanding of the development of sleep-wakefulness patterns in the infant monkey should have considerable bearing for the interpretation of behavioral dysfunctions which follow various forms of fetal and infantile brain damage. It has been suggested elsewhere that such trauma as neonatal asphyxiation affects their behavioral sequelae through a depression of directed activity and environmental reactivity. Some of the data recently collected in this laboratory, strengthen this point of view; certainly these data indicate that a more careful and detailed analysis of the infant in his environment be undertaken.

Proposed Course of the Project: The data collection from Group E (threshold determination) as well as from Group A dealing with those aspects of long-term (three month) recording sessions under laboratory and sound-shielded conditions will be completed early in the coming fiscal year (FY 1966). The subjects from Group B (deprivation of patterned vision) will be sent to the Department of Anatomy, University of California in Los Angeles for continued study. (Dr. Berger has accepted an appointment in that department and has been encouraged to pursue this investigation with the facilities there. The collaborative relation between the two principal investigators will continue, however). In so far as the fiscal operations of this laboratory are concerned, the entire project will be concluded with the analysis and publication of the data during the coming fiscal year.

Part B included: Yes.

Serial No. NDB(CF) 63-LPP 1077

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Meier, G. W., and Berger, R. J.: Development of Sleep and Wakefulness Patterns in the Infant Rhesus Monkey Macaca-mulatta. Exptl. Neurol., (in press).

Serial No. NDB(CF) 59-LPP 603
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Social behavior, reproduction, and population dynamics in free-ranging Rhesus monkeys

Principal Investigator: Dr. Carl B. Koford

Other Investigators: Dr. John G. Vandenbergh

Cooperating Units: None

Man Years:

Total:	12.3
Professional:	3.0
Other:	9.3

Project Description:

Objectives: 1. To investigate the daily, seasonal, annual and lifelong behavior of free-ranging primates, principally rhesus monkeys (M. mulatta), with emphasis on reproduction, group relations, and population dynamics.

2. To determine the influences on behavior of internal and external environmental factors.

Methods Employed: Primarily field observation of marked free-ranging rhesus monkeys on three islands. Selected animals are captured, examined, and released. Some are modified by castration, hysterectomy, implants, caging, or other procedures. Two groups are permanently held in compounds for intensive observation.

Major Findings: On Cayo Santiago, during calendar year 1964 there were 119 births, including one stillbirth. Of 139 mature females, 86% gave birth vs. 82% the previous year. Although four precocious 3-year-old females gave birth, the rate for 4-year-olds (the normal primiparous age class) was low, only 71% (vs. 74% in 1963, 100% in 1962). Of 20 females delivered by cesarean section in April, 1963, 18 gave birth without complications in 1964. One of the 20 died before pregnant and one failed to conceive, but there were no other conspicuous changes in condition or social status among the operated females.

Cayo Santiago

In 1964 the birth season was unusually compact; all births occurred within a period of 126 days. In spite of a 70% increase since 1960 in the annual number of births, the period of births has tended to decrease, in large part because of an increased tendency for all bands to breed at the same time. Among the seven bands the spread in initial birth dates was only 28 days, half of that in some previous years. The maximum rate of births was 21 in 10 days, during the first part of February. For all births (454) during a 5 year period, the highest proportion has occurred in March (38%), and next highest in February (26%).

Although 1964 was an unusually dry year, this fact did not appreciably delay mating; as in 1964, four births occurred in January, 1965. The peak of birth rate in 1965, however, occurred a month later than in the previous year; during the first 10 days of March there were 31 births. Of an anticipated 140 births, more than half (77) occurred in March, and the distribution of births (112 by April 20) was as compact as in 1964, though more peaked. There appeared to be no pregnancies among 3-year-old females.

The mortality rate of infants born in 1964 was again high (11.8%), as in 1963 (12.7%), and infant mortality so far in 1965 is even higher. This high mortality may be the indirect result of crowding, which may affect lactation, as the island population increases. During the year there were two known deaths of wounded adult males from tetanus; females are rarely so afflicted.

Excluding new infants, the island population is 452 monkeys, an increase of 14% over 1964. During the year six adolescents were removed for psychological studies of differences in sexual behavior between laboratory and island reared animals. The island population comprises seven natural bands of 142, 130, 52, 51, 40, 21, and 16 members. The last band formed during late fall by slow separation of a subgroup from the largest band accompanied by integration of three adult males, one formerly high ranking, one semisolitary, and one alien. This is the first new band formed since late in 1960.

During the year there were no changes in status of the established band leaders. The 7-year-old son of the highest ranking female continued to lead the largest band, while the former leader held second rank. The second ranking male of another band was wounded and became wasted with pneumonia; yet the larger, healthier, third ranking male made no attempt to advance in rank, apparently because the learned relation persisted.

Analysis by D. Sade of observations made in 1962-63 further indicated the importance of kinship in determining social rank.

Among infants and yearlings of the same age, social rank followed the order of rank among their mothers. When young matured they tended to rank close below their mothers and above older animals which were inferior to their mothers.

The population still includes a 2-year-old asphyxiate, four castrate males, a spontaneous castrate, and a hysterectomized female. The last was revealing in that she went through estrus and received copulations only during the normal mating season. This fact confirms the conclusion of Conaway and Koford (1964) that non-pregnant females do not cycle at other times of the year. In addition, rectal palpation of three such females during the birth season indicated that the uterus was very small, as if inactive.

A Visiting Scientist, Dr. George Bartholomew, concentrated on detailed observations of mating behavior in one band during one month of the early breeding season, and a Guest Worker, Mr. Duane Quiatt, studied the same band during two months of the early birth season. These studies will provide a fairly complete picture of year around subtle changes in social and reproductive relations.

La Parguera

By January, 1965, the population on La Cueva and Guayacan islands had increased to 173, of which 29 were in enclosures or cages and 144 were free-ranging. The latter made up five natural bands of 38, 28, 22, 18, and 17 constant members, whereas 20 individuals were occasional members of various bands. Except for an all-male band of 17, the bands have become increasingly stable, with relatively few interchanges.

Guayacan is occupied by only one band, the second largest, led since 1962 by a big Cayo Santiago male. In fall he was wounded and became somewhat weak. Two weeks later a semisolitary male joined the band and became leader. When the former leader recovered, he remained in second rank.

In 1964 there were 40 births among 45 mature females, a reproductive rate of 87%, about the same as at Cayo Santiago. Births commenced in March, with greatest numbers born in May and June (15 each). The birth season was about two and one-half months later than at Cayo Santiago, as contrasted to about four months later the previous year. In 1965, births in the free-ranging monkeys again commenced in March; only 5 of a predicted 38 occurred by mid-April. Apparently the trend toward breeding at the same time of year as the Cayo Santiago population has ceased.

In part the yearly and geographic differences in birth season may be related to rainfall through its effects on vegetation at the beginning of the wet season. At Parguera, where rainfall was only 15"

and 17" during the past two years, any such effects are probably intensified. Consequently, year around studies of the phenology of 50 marked trees and shrubs have started.

In comparison to the season of births in the free-ranging monkeys, births in the enclosures commenced a month earlier and in a large cage two months earlier. Although these captive births were few (6), they suggest that captivity outdoors can advance the annual cycle. In one enclosure a female with ligated tubes commenced estrous cycles at the same time as normal females and went through four cycles.

A second 10,000 square foot enclosure was completed and stocked with a small natural group. These animals included the one-eyed teras born in 1963, which seems socially normal. A subsequent infant born (1965) to the same mother appears to be physically normal. The new enclosure is used, in part, for development of telemetric studies of heart rate and electrocardiogram responses to social encounters. The rate for an inactively sitting monkey may vary from 85 per minute in normal dozing to 296 per minute when an alien approaches. Although so far we have only one usable transmitter, Mr. J. Rosenthal of the Technical Development Section is improving and extending the system.

Additional squirrel monkeys (Saimiri) were captured near an abandoned zoo, so that now we have seven animals including three females and a yearling male. These will be released on an isolated half-acre mangrove islet where supplementary food and water are provided, in order to develop a naturalistic free-ranging band. The caged tree shrew colony stands at six, including two females. Three of these animals were raised during the past year, but at least three other young died of apparent maternal neglect. One pair of Tupaia glis was exchanged for a pair of laboratory reared T. longipes, a more tractable species with a good reproductive record. When numbers are sufficient the animals will be established on an islet. The fact that an escaped male on La Cueva was recaptured seven months later indicates that the environment is suitable.

Significance: 1. Current studies of free-ranging populations are supplying detailed knowledge of the social dynamics, reproductive biology, and environmental relations of naturalistic groups of rhesus monkeys. For these and similar primates, such data were lacking. Reliable knowledge of this kind must be based on long term observations of large numbers of individually marked animals; in this respect our program is unsurpassed.

2. Detailed knowledge of natural behavior is needed for the conception and interpretation of experiments in the laboratory or other artificial situations. The adaptive and evolutionary significance of findings can be meaningfully judged only in natural context.

3. This program aims to solve many problems, such as the evolution of social behavior, the survival value of territoriality, the relation of kinship to social status and status to fertility, and the effects of population density on mortality and reproductive cycle, which cannot be attacked in ordinary laboratory facilities and are extremely difficult to solve by studies of completely wild populations.

Proposed Course of the Project: The present long-term studies of the entire free-ranging population, as well as short concentrated studies on limited problems in single bands and enclosures, will continue. These studies will be extended, on the one hand, to correlate with laboratory findings, especially in the fields of reproductive histology and endocrinology. On the other hand, studies will be extended to include small groups of radically different primates (tree shrews, squirrel monkeys) and also to correlate with studies of macaques in other geographic areas and under more natural conditions. To this end, it is planned to transplant a large number of animals from Cayo Santiago, where overcrowding is destroying vegetation and apparently increasing mortality, to Desecheo Island, which is much larger. This island, off the western tip of Puerto Rico, includes about 300 acres of forest and an equal area of barren slopes. Farther afield, Dr. Koford plans to take leave of absence during 1966, principally for the purpose of studying the annual reproductive cycle in several populations of wild Japanese macaques. These studies will be of special value for comparison with similar studies of rhesus monkeys in Puerto Rico.

Part B included: Yes.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications:

Adamsons, K., Behrman, R., Dawes, G.S., James, L.S. and Koford, C.B.: Rususcitation by positive pressure ventilation and Tris-hydroxymenthlaminomethane of rhesus monkeys asphyxiated at birth. J. Pediat., 65: 807-818, 1964.

Conaway, C.H. and Koford, C.B.: Estrous cycles and mating behavior in a free-ranging band of rhesus monkeys. J. Mamm., 45: 577-588, 1964.

Sade, D.S.: Seasonal cycle in size of testes of free-ranging Macaca mulatta. Folia primat., 2: 171-180, 1964.

Conaway, C.H. and Sade, D.S.: The seasonal spermatogenic cycle in free-ranging rhesus monkeys. Folia primat., 3: 1-12, 1965.

Kaufmann, J.H.: Studies on the behavior of captive tree shrews (Tupaia glis). Folia primat., 3: 50-74, 1965.

Kaufmann, J.H.: Social relations of adult males in a free-ranging band of rhesus monkeys. In "Social Interaction among Primates." S.A. Altmann (ed.), Univ. Chicago Press, 1965 (in press).

Kaufmann, J.H.: A three year study of mating behavior in a free-ranging band of rhesus monkeys. Ecology, 1965 (in press).

Koford, C.B.: Population dynamics of rhesus monkeys on Cayo Santiago. In "Primate Behavior: Field Studies of Monkeys and Apes," Irven DeVore (ed.) Holt, Rinehard and Winston, N.Y., 1965.

Koford, C.B.: Changes in the Cayo Santiago rhesus monkey population, 1960-1964. Proc. Delta Regional Primate Research Center Symposium, Nov., 1964. Folia primat. (in press).

Rowland, E. and Vandenbergh, J.G.: A survey of the intestinal parasites in a new colony of rhesus monkeys. J. Parasit., 1965 (in press).

Sade, D.S.: Dominance in a group of free-ranging rhesus monkeys. In "Social Interaction among Primates," S.A. Altmann (ed.) Univ. Chicago Press, 1965.

Vandenbergh, J.G.: Hormonal basis of sex skin in male rhesus monkeys. Gen. comp. Endocr., 5: 31-34, 1965.

Vandenbergh, J.G.: Social dynamics in a new free-ranging colony of rhesus monkeys. In "Social Interaction among Primates," S.A. Altmann (ed.) Univ. Chicago Press, 1965 (in press).

Vandenbergh, J.G.: Social differences between artificially and spontaneously formed groups of rhesus monkeys. Proc. Delta Regional Primate Research Center Symposium, Nov. 1964. Folia primat. (in press).

Serial No. NDB(CF) 63LPP 1088
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Mechanism of vasomotor response observed after i.v. administration of a muscle relaxant in the cat.

Principal Investigators: Dr. Walter L. Stiehl

Other Investigators: Dr. C. C. González
Dr. Rafael Berríos

Cooperating Units: Departments of Anatomy, Pharmacology and Neurology, School of Medicine, University of Puerto Rico San Juan, Puerto Rico

Man Years:
Total: .5
Professional: .3
Other: .2

Project Description:

Objectives: To determine the mechanism responsible for the vasomotor changes observed upon stimulation of the floor of the fourth ventricle after i.v. administration of a muscle relaxant.

Methods Employed: The same methods were employed that have been reported previously with the following modifications. Provisions were made to study separately a) the peripheral vasomotor response by measuring the isolated femoral flow in a leg and b) the cardiac phenomena by doing differential recordings from each ventricle surface.

Major Findings: A. Peripheral circulation: The pressure curves obtained were essentially similar to those reported previously. It was found that peripheral circulation diminished when the drug was given. The reduction in peripheral circulation was comparable to that produced by the injection of nor-epinephrine. Isolation of the visceral (G.I.) circulation made no significant difference. No attempt was made to separate the surface (skin) circulation from the muscle circulation. B.Cardiac Phenomena: Differential recordings from each ventricle showed (upon administration of the drug) the same increase in potential that had been observed with standard leads.

After the second dose a dissociation of the ventricular contractions

was observed with the right ventricle leading, the contractions then became isochronic and eventually the left ventricle contraction occurred before the right. After few minutes isochronicity was reestablished. A marked cardiac dilatation occurred (simultaneous with the drop in blood pressure after the drug had been administered).

Significance: A.Peripheral response: The observed reduction in the peripheral flow helps explain two puzzling previous observations. The small rise that occurs just before the blood pressure falls is due to the beginning of peripheral vasoconstriction. The rise that should follow is offset by a drop in blood pressure of cardiac origin. The marked vasoconstriction that occurs with the drug explains the observation that nor-epinephrine is not effective in preventing the drop in blood pressure caused by the drug. The fact that nor-epinephrine is not effective in raising the blood pressure after it stabilizes at a low level upon the injection of repeated doses of the drug suggests that the peripheral vasoconstriction is a sustained phenomenon. The first injection of epinephrine (due to its cardiac action) is effective in raising the blood pressure. B. Cardiac Phenomena: The results obtained by differential recording of the right and left ventricular contractions demonstrate that the increase in ECG potentials is observed on the cardiac muscle surface. The loss of isochronicity of the ventricular contraction observed with cardiac electrodes explains the change in ECG shape observed with standard leads.

Proposed Course of Project: At present we are expecting a sample of the pure drug in order to continue the study of the cardiac phenomena. We will proceed to study the effect of the drug after denervation of the heart to determine whether the effect observed in the heart is central or peripheral. Cardiac output studies will be done if found necessary.

Part B included: No

Serial No. NDB(CF) 63-LPP 1089
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Quantitative study of the growth of the skull and brain in the cat from birth to seventeen weeks of age.

Principal Investigator: Dr. Walter L. Stiehl

Other Investigators: Dr. Carmen Eva Cabrera

Cooperating Units: Harvard Dental School, Boston, Masschusetts

Man Years:
Total: .3
Professional: .1
Other: .2

Project Description:

Objectives: To establish much needed baselines regarding periodic increment in the three planes and the time when maximum size is attained in the skull of the cat. To determine the relative position of brain structures to skull landmarks at different ages during growth of the skull.

Methods Employed: Animals of known age are sacrificed at relative intervals of one week. The head, neck and upper extremities are perfused through the aorta with 10% formaline-saline solution. The heads are then mounted in a Horsley-Clark apparatus and the coordinates determined for two sets of pre-determined points, 25 points are measured on the skull and 63 on the brain. The points obtained are then plotted separately for each plane against the logarithm of the age.

Major Findings: Work has been finished for the skull measurements in the first group of animals. A second group is being collected at present. From the data obtained it can be seen that the position of the internal auditory meatus changes relatively little during growth in relation to the external auditory meatus. Anterior to this point the bone grows at a successively faster rate as one moves from the middle to the anterior cranial fossa and then to maxillo-facial structures.

The highest rate of growth is attained by the maxilla and the mandible. A similar pattern, but with a slower rate of growth, is seen posterior to the internal auditory meatus. The rate of growth in the lateral and vertical directions is much slower than in the antero-posterior direction. All growth curves begin to flatten out at the tenth or eleventh weeks. Comparison with average measurements of adult animals shows that maximum size for the cranium is attained around this age.

Significance: The information obtained will not only provide a quantitative description of the growth of the skull and brain in the cat but will also provide information regarding the spatial orientation during growth of different portions of the brain.

Proposed Course of the Project: To continue the collection and measurement of a new group of animals. After measurements are completed this information will be added to that already available and new growth curves will be drawn.

Part B included: No

Serial No. NDB(1) 65-LPP 1266

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The circulation of the lungs.

Principal Investigators: Dr. Walter L. Stiehl

Other Investigators: None

Cooperating Units: Department of Anatomy, School of Medicine, University of Puerto Rico, San Juan, Puerto Rico.

Man Years:

Total:	.7
Professional:	.4
Other:	.3

Project Description:

Objectives: To study the frequency of occurrence of vascular patterns in human bronchial and pulmonary circulations.

Methods Employed: This project involved a review of the pertinent literature since 1941 plus the detailed dissection of six corrosion preparations of human plastic injected bronchial, arterial and venous trees to determine the frequency incidence of different patterns of vascularization to the segment of the lungs.

Major Findings: It was found that the dissections done corroborated the findings reported in the literature since 1941 and showed a considerable variance with the information presented in the classical textbooks.

Significance: The work showed that a considerable lag exists between the results of recent investigations and textbook presentations.

Proposed Course of the Project: None.

Part B included: Yes.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Schobinger, R., and Ruzicka, F. (Eds.): Vascular Roentgenology, Arteriography Venography Lymphangiography, 1st. ed. New York, The MacMillan Co., 1964.

Serial No. NDB(I) 65-LPP 1267
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The effect of temperature change on muscle activity in the extremities after upper motor neuron lesions.

Principal Investigators: Dr. Walter L. Stiehl
Dr. Rafael Berríos

Other Investigators: Dr. C. C. González

Cooperating Units: Departments of Anatomy, Pharmacology and Neurology, University of Puerto School of Medicine, San Juan, Puerto Rico

Man Years:
Total: .5
Professional: .2
Other: .3

Project Description:

Objectives: To study the change that occurs in muscle activity upon application of thermal stimuli, after upper motor neuron damage.

Methods Employed: Myography has been carried out in patients showing complete transection of the cord, transverse myelitis or higher central lesions that result in a similar paralysis. Myography needles are inserted to record from a superficial muscle. The temperature of the area is then lowered or increased by using cold or warm applications while a continous monitor of the myography is being done.

Major findings: The patients observed show as a constant control condition a low level of spontaneous unit firing with irregular intervals and low voltages. Upon application of a cold stimulus the rate increases markedly and the voltages become much larger. The application of a warm stimulus results in a decrease in frequency and lower voltages. Monitor of deep-muscle temperatures has not been done.

Significance: One of the investigators has reported previously (See 1964 Report) the results obtained by changing the temperature on preparations of the extensor of the fourth toe in Rana pipiens and the common

digital extensor in Bufo Marinus. It was suggested that these results were due to the spontaneous liberation of ACH at or near the muscle spindle with increase in temperature. Due to the known fact that temperature in the extremities decreases when exposed to a cold environment, it was further suggested that temperature sensation afforded by this mechanism may help in local adjustments of muscle activity that will help to offset the loss of heat. The present investigation shows that upon the application of local thermal stimuli there is a similar change in muscle activity that tends to offset the effect of the stimulus.

This phenomenon has been observed in all patients examined including one where surgical removal of lumbar and upper sacral levels had resulted in expensive atrophy of lower extremity musculature except for the lateral head of the gastrocnemius where the myography was done.

Proposed Course of Project: It is intended to repeat the examinations in patients after local anesthesia has abolished thermal responses arising from the skin. Studies will also be done in the laboratory using cats where a previous transection of the cord has been carried out.

Part B included: No

Serial No. NDB(I) 65-LPP 1265
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Responsiveness of denervated diaphragmatic muscle to immunological stimulation.

Principal Investigators: Dr. F. Alonso de Florida
Dr. José del Castillo

Other Investigators: None

Cooperating Units: Department of Pharmacology, University of Puerto Rico School of Medicine, San Juan, Puerto Rico

Man Years:
Total: 1.7
Professional: 1.2
Other: .5

Project Description:

Objectives: To study the immunological responsiveness of denervated diaphragmatic muscle of the guinea pig.

Methods Employed: Antigenic stimulation of guinea pigs; dissection of diaphragmatic muscle with in vitro study in sucrose gap chamber; microelectrode investigation of muscle fiber response to stimulation with protein antigen.

Major findings: The denervated diaphragm of the guinea pig becomes sensitive not only to acetylcholine but exhibits also marked sensitivity to histamine and bradysinin. Further, denervated diaphragm taken from immunized guinea pigs contract in the presence of small concentrations of homologous antigen. This response elicited by an antigenic protein appears to be of immunological origin showing the characteristic of the Schultz-Dale reaction of visceral smooth muscle. The contractions are observed only in diaphragmatic muscle taken from actively or passively immunized guinea pigs; they are elicited only by the homologous antigen or related proteins; repeated antigenic administration causes disensitization; and, finally, the dose response curves are bell-shaped showing a maximum peak instead of reaching saturation levels.

Intracellular electrodes reveal that muscular contraction elicited by antigenic stimulation is a true contraction produced by long volleys of action potentials lasting for many seconds. Such rythmic discharges are

not due primarily to depolarization but rather to an electrical instability of the membrane.

Significance: The experiments show that denervated guinea pig diaphragm develops new receptors to compounds which are inactive before degeneration of the phrenic nerves. Such a preparation offers an opportunity to investigate the mechanisms by which motor nerve fibers control the chemical sensitivity of the membrane of the muscle. It also represents a satisfactory preparation for the study of the nature of the anaphylactic response itself in terms of membrane alteration and of electrical events at the membrane level.

Proposed Course of the Project: The surface membranes of denervated muscle fibers become sensitive to low frequency, low energy pressure waves in the surrounding medium. It is proposed to investigate the implications of this fact for muscle contraction and for the electrical events of the muscle fiber membrane.

Part B included: Yes.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Alonso de Florida, F., del Castillo, J., González, C. C., and Sánchez, V.: Anaphylactic reaction of denervated skeletal muscle in the Guinea pig. Science, 147: 1155-1156, 1965.

Alonso de Florida, F., del Castillo, J., González, C. C., and Sánchez, V.: On the pharmacological and immunological responsiveness of denervated skeletal muscle of the Guinea pig. Brit. J. Pharmacol. (In press).

Serial No. NDB(CF) 62-LPP 953

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Experimental Rubella in Monkeys

Principal Investigator: Dr. John J. Sever

Other Investigators: Dr. Gilles Monif
Dr. Wendell H. Niemann

Cooperating Units: Section of Virology, PRB, NINDB
Project No. NDB (CF) 62 PR/IR 972

Man Years:

Total:	1.2
Professional:	.2
Other:	1.0

Part B included: No.

Serial No. NDB(I) 65LPP 1263
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Ocular Pathology and Experimental Allergic Encephalomyelitis in Young Monkeys.

Principal Investigators: Dr. Ludwig von Sallmann
Dr. Edwin M. Lerner
Dr. Sanford H. Stone

Other Investigators: Dr. Ronald E. Myers
Dr. Wendell H. Niemann

Cooperating Units: Laboratory of Immunology, NIAID
Laboratory of Germ-Free Animals Research, NIAID
Laboratory of Ophthalmology, NINDB
Project No. NINDB (I) 65-0-Ch 1212C

Man Years:
Total: 1.2
Professional: .2
Other: 1.0

Part B included: No

Serial No. NDB(I) 65LPP 1264
1. Intramural Research
2. Laboratory of Perinatal Research
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Studies on Auto-immune Diseases and Related Immunological Processes

Principal Investigators: Dr. Sanford H. Stone
Dr. Edwin M. Lerner

Other Investigators: Dr. Ludwig von Sallmann
Dr. R. E. Myers
Dr. Wendell H. Niemann

Cooperating Units: Laboratory of Immunology, NIAID
Laboratory of Germ-Free Animal Research, NIAID
Project No. NIAID-117

Man Years:
Total: 1.2
Professional: .2
Other: 1.0

Part B included: No

Serial No. NDB(CF) 61-LPP 823

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Neurologic and Electroencephalographic Studies of Monkeys Brain Damaged by Asphyxiation During Birth.

Principal Investigator: Dr. O. Esquivel de Gallardo

Other Investigators: Dr. María I. Robert de Ramírez de Arellano
Dr. William F. Windle

Project Terminated: Investigators no longer associated with the Laboratory.

Serial No. NDB(CF) 61-LPP 824

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Hemidecortication in monkeys. Comparison of rate and degree of recovery of neurological deficit as related to age at time of operation.

Principal Investigator: Dr. O. Esquivel de Gallardo

Other Investigators: Dr. Murphy C. Combs
Dr. Max Ramírez de Arellano

Project Terminated: Investigators no longer associated with the Laboratory.

Serial No. NDB(CF) 62-LPP 949
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The EEG of monkey fetuses (Macaca mulatta) in utero from chronically implanted electrodes.

Principal Investigator: Dr. O. Esquivel de Gallardo

Other Investigators: Dr. Robert W. Fleischman
Dr. M. I. Robert de Ramírez de Arellano

Project Terminated: Investigators no longer associated with the laboratory.

Serial No. NDB(CF) 61-LPP 825

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Experimental Hyperbilirubinemia and Kernicterus in Newborn Rhesus Monkeys.

Principal Investigator: Dr. Richard E. Behrman

Other Investigators: Dr. Jerold Lucey
Dr. Emerson Hibbard
Dr. Roger Lester
Dr. William F. Windle
Dr. O. Esquivel de Gallardo

Project Terminated: Investigators no longer associated with the laboratory.

Serial No. NDB(CF) 59-LPP 602

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Asphyxia in the newborn rhesus monkey; study of effects of rapid alkali and glucose infusion, of respiratory stimulants, and of rapid cooling.

Principal Investigator: Dr. Geoffrey Dawes

Other Investigators: Dr. L. Stanley James
Dr. Benjamin Ross
Dr. John J. Schruefer

Project Terminated: Investigators no longer associated with the laboratory.

Serial No. NDB(CF) 62-LPP 950

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Fetal water and electrolyte changes and amniotic fluid changes with gestation and in the presence of artificial osmotic gradients. In vivo uterine arteriovenous differences of glucose and oxygen and in vitro permeability of specific placental membranes at different stages of gestation.

Principal Investigator: Dr. Frederick C. Battaglia

Other Investigators: Dr. Paul Bruns
Dr. André E. Hellegers
Dr. A. Elmore Seeds
Dr. Richard E. Behrman

Project Terminated: Investigators no longer associated with the laboratory.

Serial No. NDB(CF) 58-LPP 502

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Histopathology of the Central Nervous System of Macaca mulatta after Asphyxia Neonatorum. Behavioral-anatomical relations.

Principal Investigators: Dr. William F. Windle
Dr. Geoffrey Dawes

Other Investigators: Dr. Emerson Hibbard
Dr. Gilbert W. Meier

Project Terminated: Investigators no longer associated with the laboratory.

Serial No. NDB(CF) 61-LPP 826

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Regeneration in the central nervous system of ammocoetes.

Principal Investigator: Dr. Emerson Hibbard

Other Investigators: Dr. W. F. Windle

Project Terminated: Investigators no longer associated with the laboratory.

Serial No. NDB(CF) 62-LPP 951
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Serial duplication grafts of hindbrain in frog embryos.

Principal Investigator: Dr. Emerson Hibbard

Other Investigators: None

Project Terminated: Investigators no longer associated with the laboratory.

Serial No. NDB(CF) 62-LPP 952

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Vascular supply to the brain of the larval lamprey.

Principal Investigator: Dr. Emerson Hibbard

Other Investigators: None

Project Terminated: Investigators no longer associated with the laboratory.

Serial No. NDB(CF) 63-LPP 1078

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Tumors induced in the monkey (Macaca mulatta) by Rous chicken sarcoma virus.

Principal Investigator: Dr. J. Spencer Munroe

Other Investigators: Dr. William F. Windle
Dr. M. I. Díaz Faro
Dr. Phillip Farber

Project Terminated: Investigators no longer associated with the laboratory.

Serial No. NDB(CF) 63-LPP 1079

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Measurement of Uterine Blood Flow in the Pregnant Rhesus Monkey (Macaca mulatta). Further studies on Fetal Water Metabolism.

Principal Investigator: Dr. Donald H. Barron

Other Investigators: Dr. Giacomo Meschia
Dr. Frederick C. Battaglia
Dr. André E. Hellegers
Dr. Richard E. Behrman
Dr. John J. Schruefer

Project Terminated: Investigators no longer associated with the laboratory.

Serial No. NDB(CF) 63-LPP 1084

1. Intramural Research
2. Laboratory or Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Chromosome studies on Macaca mulatta utilizing the leucocyte culturing technique.

Principal Investigator: Dr. Phillip A. Farber

Other Investigators: None

Project Terminated: Investigators no longer associated with the laboratory.

Serial No. NDB(CF) 63-LPP 1085

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Regional blood-flow in the brain of rhesus monkeys: Relation to age.

Principal Investigators: Dr. Seymour S. Kety
Dr. Louis Sokoloff
Dr. Charles Kennedy

Other Investigators: Dr. M. I. Díaz Faro
Dr. William F. Windle

Project Terminated: Investigators no longer associated with the laboratory.

Serial No. NDB(CF) 63-LPP 1086
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The role of ACH in temperature reception by the muscle spindles.

Principal Investigators: V. Sánchez
Dr. Walter L. Stiehl

Other Investigators: None

Project Completed

Serial No. NDB(CF) 63-LPP 1087
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The response of muscle spindles to local changes in pH.

Principal Investigators: Dr. Walter L. Stiehl
V. Sánchez

Other Investigators: Dr. M. Avilés

Project Completed

ANNUAL REPORT
JULY 1, 1964 THROUGH JUNE 30, 1965
ASSOCIATE DIRECTOR'S REPORT
COLLABORATIVE AND FIELD RESEARCH
NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

On February 1, 1965, William F. Caveness, M.D., entered on duty as the Associate Director for the Collaborative and Field Research Program, NINDB. Prior to that time Richard L. Masland, M.D., Director, NINDB, served as Acting Associate Director for C&FR.

The year has been most productive. Research accomplished has been of high quality within the limitations of positions and funds allocated to this program area. Fiscal Year 1966 will be even more successful. Summary of significant events of this year are as follows:

1. The Model Reporting Area for Blindness Statistics of the Biometrics Branch saw two new members admitted thereby increasing the total membership to 12 member states. Five other states are under contract engaging their efforts to updating their states' registers. These 17 States represent approximately one-third of the total U. S. population. This collaborative statistical project serves as a means for wide-scale data on prevalence, incidence, visual acuity, and cause of blindness is made available. It is the only data of its kind. With increased growth predictable, statistics on blindness in the U. S. will be significantly useful in planning programs of prevention and control. The MRA program has received wide support and endorsement throughout the country. Interest in establishing a similar project has been experienced from countries abroad such as Egypt, India, and Israel. The World Health Organization at Geneva and the Royal Commonwealth Society for the Blind in London have also expressed interest in the development of the MRA.

Commendations have been received from the National Council of State Programs for the Blind, the American Optometric Association, the Council of State Directors of Vocational Rehabilitation, and the American Academy of Ophthalmology and Otolaryngology.

2. Epidemiology Research Program -- In October, a site visit was made by a four member team to the Epidemiology Branch's Research Center, Agana, Guam to appraise the current status of its programs and its projects and at the same time to acquaint the new Branch Chief, Dr. Jacob A. Brody, who will assume the position of Chief, Epidemiology Branch, NINDB on July 1, 1965, (on November 1, 1965, Dr. Leonard T. Kurland, Chief, Epidemiology Branch, retired from the Commissioned Corps) and Dr. William F. Caveness, the new Associate Director, C&FR, with the research program there. It was the

recommendation of the review team that value of the clinical and pathological resources on Guam be recognized as a lasting importance and that the future of the Center's activities and program focal point should be the etiological factors including nutritious and infectious agents.

Upon Dr. Kurland's retirement, Dr. John T. Schwartz served as Acting Chief, Epidemiology Branch. Program emphasis under Dr. Schwartz, an ophthalmologist, was directed to the development and establishment of a collaborative and field research program of epidemiological investigation of eye disorders. These initial efforts which were begun for a vision program in epidemiology will be implemented on a large-scale basis during Fiscal Year 1966.

3. Special Projects - Head Injury -- With the recognition of the magnitude of the problem of cranio-cerebral trauma and that it warrants top priority for program planning, a Head Injury Planning Committee was established. This 9 member committee, composed of experienced neurologists, neurosurgeons, and physiologists, held four meetings, January 5, February 5, February 27 and May 1, 1965, to determine format and objectives for a conference on Head Injury. This conference is scheduled for February 7 - 9, 1966 at the University of Chicago.

The objectives are defined as follows:

(1) A comprehensive evaluation of the present state of knowledge concerning head injuries and the principal investigation now being conducted in this field.

(2) Categorization of head injuries.

(3) Recommendations as to the most promising areas, clinical and laboratory, for further investigation of the acute phase of injury.

(4) Recommendations for the most effective methods for the study of post-traumatic sequelae.

This conference has the endorsement of the American Neurological Association, the American Physiological Society, and the Society of Neurological Surgeons. This conference will be supported through a grant to the University of Chicago.

From this conference's recommendations, a freshly conceived clinical and basic investigation will be undertaken.

4. Special Projects - Epilepsy Program -- Concern over epilepsy as a major health problem and that little demonstrated progress in the areas of drug therapy, surgical treatment, electroencephalography, and basic research over the past two to three decades spirited NINDB to

develop a sound clinical research program for the study of epilepsy. Initial steps, this year, through a workshop on epilepsy has produced a collaborative effort between four clinical centers and a coordinating and data processing unit. Their efforts initially will be to pre-test and refine a protocol over an 18 month period. There will follow a full scale clinical research program if this joint project phase is successful. As part of the full scale phase, an intensive drug development evaluation program involving the resources of both university and industrial pharmacological facilities and an electronic and microchemical instrumentation development program will be instituted.

The Office of the Secretary, DHEW, has expressed a deep concern on this problem of epilepsy. This office held a meeting in April 1965 establishing a Task Force on Epilepsy to identify gaps in knowledge, major policies, problems and barriers at local, state, and national level, which impede progress in needed research, service and professional training aspects of epilepsy in relation to health, education, and welfare. This meeting's further purpose was to suggest possible courses of action at the national level which would help overcome barriers to progress in the prevention and control of epilepsy.

This meeting has served as an additional incentive to the clinical research program on epilepsy planned by this program area of this Institute for the conquest of this disease.

The Task Force has suggested that NINDB and BSS (Division of Chronic Diseases) establish soon an Advisory Panel on Epilepsy representative of the disciplines with subdivisions to be responsible to each agency. This panel will advise in the pursuit of the epilepsy problem. This panel while of particular value to NINDB and BSS will be of service to the DHEW in its national epilepsy program.

Fiscal Year 1966 will be devoted to strengthening these programs in the C&FR area, and to exercising vigorous energy in the accomplishment of each and every objective. Realistic budgetary support is expected. Any delays in this regard will have its toll on program accomplishments.

Serial No. NDB(CF)65-OAD 1282 (I-IX)
1. Collaborative-Field Research
2. Office of Associate Director
3. Bethesda, Maryland

Projects NDB(CF)-62 OAD 970, NDB(CF)-63 OAD 1138 and 1139 have now been integrated into this project.

PHS-NIH
Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Studies of Child Growth, Development, and Behavior, and Disease Patterns in Primitive Cultures

Sub Project I: Study of the Developmental Patterning of the Human Nervous System (A Cybernetics of Human Development)

1. A research archive for ethnopediatric film investigation of styles in the patterning of the nervous system.
2. Analysis of child care and behavior patterns in primitive cultures from photographic recording (development of techniques and methods).
3. Investigation of non-recurrent phenomena (objectives and selectivity to be used in documentation of aperiodic phenomena to preserve maxiumum information).
4. Analysis of culturally determined methods of approach to symbolic representation from drawings and art forms of children and adults in primitive societies.

Sub Project II: Human Evolutionary Study in Isolated Primitive Groups

1. Kuru.
2. Motor neuron disease and other degenerative diseases in New Guinea and in other inbred Pacific Island populations.
3. Blood group genetic study in Australasian (Melanesian and Micronesian) and South American indigenous groups.
4. Studies on polymorphism of hemoglobins, red cell enzymes, and serum proteins in Australasian (Melanesian and Micronesian) and South American indigenous groups.

Sub Project III: Studies of Isolated Micronesian Populations

1. Child development and behavior on Ulithi, Ifaluk and Lamotrek atolls and Fais Island.
2. Response to live measles virus vaccine in immunologically virgin populations without circulating measles virus (with special attention to response in susceptible adults and pregnant women and their offspring).
3. Influenza A_2: virgin-soil epidemics (epidemiological, clinical and immunological response and discovery of populations without previous experience with Type A or Type B influenza).

4. Study of infectious disease patterns in remote individual island populations.

5. Genetic characterization of the population of the Caroline Islands.

Sub-Project IV: Studies of Isolated New Guinea Populations

1. Child development and behavior in the Western Dani, Asmat, Tjitjak, Auyu, Kayagar groups of West New Guinea, and the Kukukuku and Eastern Highland people of Territory of Papua and New Guinea, the Mangsing, Mamusi and West Nakanai of New Britain.

2. Study of infectious disease in the diverse ecologically isolated New Guinea populations.

3. Genetic characterization of New Guinea populations.

4. Hereditary and genetic disease patterns in New Guinea.

5. The Kukukuku peoples: an intensive longitudinal study of child growth, development and behavior, and disease patterns, human genetics and communication in an archaic Highland population of New Guinea.

6. Research cinema films on behavioral patterns of children in New Guinea cultures.

Sub-Project V: Studies in Isolated New Hebridean and Solomon Islands Populations

1. Child development and behavior on Tongariki and the Banks and Torres Islands and with the Ipayato of Espiritu Santo.

2. The Tongariki project: an intensive study of human evolution in the Shepherd Islands.

3. Human genetics and disease patterns survey of the Banks and Torres Islands.

4. Infectious disease seroepidemiology of the New Hebrides.

Sub-Project VI: Studies on Australian Aborigines

1. Arbor virus seroepidemiological studies on Australian aboriginal communities of Cape York.

2. Infectious disease patterns survey of aborigines of Bentnik-Mornington Islands, the Kimberly, the Haast's Bluff and Cape York regions of Australia.

Sub-Project VII:Studies on Central and South American Indians

1. Human genetics and disease pattern studies of the Guayaki and Chaco Indian tribes and of the Mennonite colonists in the Chaco of Paraguay.

2. Children of the Guayaki Indians of Southeastern Paraguay.
3. Children of the Ayore (Moro) Indians of Bolivia and Paraguay.

Sub-Project VIII: Developmental, Genetic and Disease Pattern Studies in Other Primitive Populations of Asia, Africa and Polynesia.

Sub-Project IX: Experimental Developmental Neuro-pediatrics or Infantile Programming:an Empirical Approach to the Languages of Information Input into the Nervous System.

Principal Investigator : D. Carleton Gajdusek, M.D.

Other Investigators: Paul Brown, M.D., Michael Alpers, M.D., Frank D. Schofield, M.D., C.J. Gibbs, Jr., Ph.D., Nancy Rogers, E. Richard Sorenson, Mint Basnight, Michael Nicholson

Technical Assistants: Judith Shaw

Student Assistants: Vicky Bigish, Leo Bourne, Peter Fetchko, John Green, Patricia Hunt, Arthur McCausland, Joseph Morris, Joanne Samuelson, Erica Weinstein

Man Years (computed for 12 month period)

Total:(including secret.)	9.5
Professional (3 GS 9)	3.7
Others	5.8

Part B included: Yes

Part B: Publications

Gajdusek, D.C.: Ethnopediatrics as a study of cybernetics of human development. The use of primitive cultures as field laboratories for the study of the programming of the nervous system of the child. Am. J. Dis. Child., 105: 554-559, 1963.

Sorenson, E.R. and Gajdusek, D.C.: The investigation of non-recurring phenomena. The research cinema film. Nature, 200: 112-114, 1963.

Neumann, Meta A. and Gajdusek, D.C.: Neuropathologic findings in exotic neurologic disorders among natives of the Highlands in New Guinea. J. Neuropath. Exp. Neurol., 23: 189, 1964.

Neumann, Meta and Gajdusek, D.C.; and Zigas, V.: Neuropathologic findings in exotic neurologic disorders among natives of the Highlands of New Guinea. J. Neuropath. Exp. Neurol., 23: 486-507, 1964.

Gajdusek, D.C., Kirk, R.L. and Guiart, J.: Familial periodic paralysis with complete heart block in a family of New Hebridean (Melanesian)-Australian aboriginal crossing. First report in aboriginal Australasians. Program and Abstracts Am. Ped. Soc., 74th Ann. Mtg., Seattle, June 16-18; 137, 1964.

Gajdusek, D.C.: Sex avoidance and pederasty with juvenile fellatio as traditional homosexuality among bisexual southwestern Kukukuku people in New Guinea. Program and Abstracts of Am. Ped. Soc., 74th Ann. Mtg., Seattle, June 16-18, 137-138, 1964.

Gajdusek, D.C.: Congenital absence of the penis in Muniri and Simbari Kukukuku people of New Guinea. Program and Abstracts of Am. Ped. Soc., 74th Ann. Mtg., Seattle, June 16-18, 138, 1964.

Gajdusek, D.C.: The composition of musics for man. Or decoding from primitive cultures the scores for human behavior. Pediatrics, 34: 84-91, 1964.

Gajdusek, D.C. and Gibbs, C.J., Jr.: Attempts to demonstrate a transmissible agent in kuru, amyotrophic lateral sclerosis and other subacute and chronic nervous system degenerations of man. Nature, 204: 257-259, 1964.

Part B: continued

Wisseman, C.L., Jr., Gajdusek, D.C., Schofield, F.D. and Rosenzweig, E.C.: Arthropod-borne virus infections of aborigines indigenous to Australasia. A preliminary report. Bull. W.H.O., 30: 211-219, 1964. Also in: Papua and New Guinea Med. J., March, 1965.

Simmons, R.T., Graydon, J.J., Gajdusek, D.C., Schofield, F.D. and Parkinson, A.D.: Blood group genetic data from the Maprik area of the Sepik District, New Guinea. Oceania, 35: 218-232, 1965.

Gajdusek, D.C., Brown, P. and Morris, J.A.: Influenza A2 epidemic in isolated Pacific atoll populations with no previous experience with prototype or contemporary A2 or contemporary B strains. Program and Abstracts Soc. Ped. Res., 35th Ann. Mtg., Seattle, May 4-6, 92, 1965.

Simmons, R.T., Graydon, J.J., Gajdusek, D.C. and Brown, P.: Blood group genetic variations in natives of the Caroline Islands and in other parts of Micronesia. With a supplement by S.H. Riesenberg: Table of voyages affecting Micronesian islands. Oceania, in press.

Simmons, R.T. and Gajdusek, D.C.: A blood group genetic survey of children of Bellona and Rennell Islands (B.S.I.P.) and certain northern New Hebridean islands. Oceania, in press.

Curtain, C.C., Gajdusek, D.C., Kidson, C., Gorman, J., Champness, L. and Rodrigue, R.: A study of the serum proteins of the peoples of the Territory of Papua and New Guinea. Am. J. Trop. Med. Hyg., in press.

Brown, P., Basnight, M. and Gajdusek, D.C.: Measles immunization of susceptible island populations in Micronesia. Am. J. Epid., in press.

Sorenson, E.R. and Gajdusek, D.C.: The study of child behavior and development in primitive cultures. A research archive for ethnopediatric film investigations of styles in the patterning of the nervous system. Pediatrics, in press.

Curtain, C.C., Gajdusek, D.C., Kidson, C., Gorman, J., Champness, L. and Rodrigue, R.: Haptoglobins and transferrins in Melanesia: Relation to hemoglobin, serum haptoglobin and serum iron levels in population groups in the Territory of Papua and New Guinea. Am. J. Phys. Anthrop., in press.

Part B: continued

Curtain, C.C., Baumgarten, A., Gorman, J., Kidson, C., Champness, L., Rodrigue, R. and Gajdusek, D.C.: Cold haemagglutinins: unusual incidence in Melanesian populations. Brit. J. Haemat., in press.

Curtain, C.C., Gajdusek, D.C., Kidson, C., Gorman, J., Champness, L. and Rodrigue, R.: Serum pseudocholinesterase levels and variants in the peoples of Papua and New Guinea. Am. J. Trop. Med. Hyg., in press.

Alpers, M. and Gajdusek, D.C.: Changing patterns of kuru: epidemiological changes in the period of increasing contact of the Fore people with western civilization. Am. J. Trop. Med. Hyg., in press.

Beck, Elisabeth, Daniel, P.M. and Gajdusek, D.C.: A comparison between the neuropathological changes in kuru and in scrapie, a system degeneration. To be presented at the Vth Inter. Congr. Neuropath., Zurich, Aug. 30-Sept. 3, 1965.

Gajdusek, D.C.: Adolescence in primitive cultures. Presented at the New York Acad. Med. (Pediat. Div.), Nov. 12, 1964.

Alpers, M.P.: Kuru. In: Manual of Trop. Med., IVth Edit. Hunter, G.W., Frye, W.W. and Swartzwelder, J.C., Editors, W.B. Saunders Co., in press, 1965.

Reid, Lucy H. and Gajdusek, D.C.: Studies on kuru. VII. A nutritional evaluation of the Moke Fore, 1957-1958. Am. J. Trop. Med. Hyg., in press.

Gajdusek, D.C.: Kuru in New Guinea and the origin of the NINDB study of slow, latent and temperate virus infections of the nervous system of man. In: Slow, Latent and Temperate Virus Infections, D.C. Gajdusek and C.J. Gibbs, Jr., editors; NINDB, National Institutes of Health, U.S. Government Printing Office, Washington, D.C., 1965.
(Monograph based on the Workshop and Symposium on Slow, Latent and Temperate Virus Infections, National Institutes of Health, December 7-9, 1964)

Alpers, M.: Epidemiological changes in kuru, 1957 to 1963. Loc cit.

Part B: continued

Gibbs, C.J., Jr. and Gajdusek, D.C.: Attempt to demonstrate a transmissible agent in kuru, amyotrophic lateral sclerosis, and other subacute and chronic progressive nervous system degenerations of man. Loc cit

Gajdusek, D.C., Zigas, V., Price, A.V.G. and Alpers, M.: Studies on kuru. VIII. Laboratory studies on kuru. In preparation.

Gajdusek, D.C., Zigas, V., Price, A.V.G. and Alpers, M.: Studies on kuru. IX. Antibody patterns in kuru patients and in residents of the kuru region and in surrounding populations. An introduction to a collaborative study. In preparation.

Simmons, R.T., Gajdusek, D.C. and Alpers, M.: Blood group genetic patterns in population isolates in and adjacent to the kuru region, Eastern Highlands, New Guinea. In preparation.

Gajdusek, D.C., Alpers, M., Curtain, C. and Steinberg, A.G.: Human genetics of population isolates in and adjacent to the kuru region, Eastern Highlands, New Guinea. In preparation.

Part A

Project Title: Studies of Child Growth, Development and Behavior, and Disease Patterns in Primitive Cultures

Sub-Project I: Study of the developmental patterning of the human nervous system (A cybernetics of human development)

1. A research archive for ethnopediatric film investigation of styles in the patterning of the nervous system.

Principal Investigators: E. Richard Sorenson and D. Carleton Gajdusek, M.D.

Other Investigators: Michael Alpers, M.D. and Judith Shaw

Cooperating Investigators: Mr. and Mrs. Jablonko, Columbia University, New York; Dr. Margaret Mead, Museum of Natural History, New York; Dr. M. Stirling, Smithsonian Institution, Washington.

Student Assistants: Fred MacMurray, Arthur McCausland, Erica Weinstein

Project Description:

The holdings of the Research Film Archive have been increased by the development of forty further research films made in connection with studies under way in this section, and by the deposit of forty-seven Research Films by collaborating depositers and investigators. Total holdings of the Archive now approximate 300,000 feet. These films have been catalogued and indexed, in accordance with the procedures previously worked out and published, as research records of non-recurring events. The continuing development of the Research Film Archive as a laboratory for the study of child growth and development and disease patterns in primitive cultures has been aimed at providing facilities for the holding and use of the Research Films and their related data which pertain to our investigations on the patterning of the human nervous system in diverse primitive cultural isolates. It is already providing a place where Research Films and Special Films are indexed, catalogued, and preserved, together with associated data in the form of field notes, journals, pertinent related literature, tape recordings, still photographs, and collections of maps, charts, genealogies, medical histories, drawings, and the like; and it is providing research workers and our collaborative investigators with facilities for analysis, study, and preparation of filmed data in relation to other data developed in our laboratories and elsewhere.

Part B included: Yes

Part B: Publications

Sorenson, E.R. and Gajdusek, D.C.: The investigation of nonrecurring phenomena. The research cinema film. Nature, 200: 112-114, 1964.

Sorenson, E.R. and Gajdusek, D.C.: The study of child behavior and development in primitive cultures. A research archive for ethnopediatric film investigations of styles in the patterning of the nervous system.

I. Advantages of the methods for the study of childhood in primitive societies in elucidating the languages of sensory input operating in the programming of the human nervous system.

II. History of scientific motion pictures.

III. The motion picture in the preservation of nonrecurring ethnoenvironmental data:

The Research Cineam Film. A detailed method of organizing motion picture film for maximum preservation of its data.

The Research Film Archive. A laboratory for the investigation of cinema recorded behavior.

The Special Film. Specific studies and investigative results based on research film.

IV. Research films for the study of child growth and development and disease patterns in primitive cultures.

Part A

Project Title: Studies of Child Growth, Development and Behavior, and Disease Patterns in Primitive Cultures

Sub-Project I: Study of the developmental patterning of the human nervous system (A cybernetics of human development)

2. Analysis of child care and behavior patterns in primitive cultures from photographic recording (development of techniques and methods)

Principal Investigators: D. Carleton Gajdusek, M.D. and E. Richard Sorenson

Project Description:

With the overall aim of elucidating the languages of sensory input operating in the programming of the human nervous system, we have developed a detailed method of organizing motion picture film for maximum preservation, retrieval, and use of its data by means of special indices and abstracts, and by use of stop frame, variable speed, and analytical viewing equipment.

A human culture exposes the infant developing within it to different messages from the elements in his environment. For example, whereas the Guayaki child lives and sleeps under the open sky without roof or walls and exposed to the caresses of breeze, and buffets of tempest, the Navajo or Hopi child is wall and roof-enclosed much of the time from early infancy; and whereas the Fore infant is afforded physical contact with his mother whenever he desires, children in many other cultures are not. An unsteady floor of loose split bamboo poles in a tree house high above the ground provides different exposure to sun, wind, rain, and temperature; and the stability of its surface provides different problems of balance and induces different dependence upon foundation than that of stone or earth floors. We are looking at and selecting such aspects of behavior and milieu bearing on the patterning of the nervous system of the developing child, such as those provided by fondling, close bodily contact, nursing, feeding, carrying postures, swaddling, rocking, sleeping, grooming, genital handling, pacifying, attention to crying, sucking, encouragement of walking or talking, enforcement of modesty, language and gesture, singing, associated movements of speech, styles of sitting and gait, mannerisms associated with emotional tension, modes of skilled motor performance, and many other aspects of behavior in a culture.

We cannot yet fully formulate the manner in which movement and styles of motor behavior can be dissected, sorted, and classified, and it would still be rash to guess at the full significance to the developing nervous system of the odd, unusual, or unique patterns of learning or fashions of environmental and cultural programming to brain function.

Part B: Publications

Gajdusek, D.C.: Ethnopediatrics as a study of cybernetics of human development. The use of primitive cultures as field laboratories for the study of the programming of the nervous system of the child. Am. J. Dis. Child., 105: 554-559, 1963.

Gajdusek, D.C.: The composition of musics for man. Or decoding from primitive cultures the scores for human behavior. Pediatrics, 34: 84-91, 1964.

Sorenson, E.R. and Gajdusek, D.C.: The Study of child behavior and development in primitive cultures. A research archive for ethnopediatric film investigations of styles in the patterning of the nervous system. I. Advantages of and methods for the study of childhood in primitive societies in elucidating the languages of sensory input operating in the programming of the human nervous system. Pediatrics, in press.

Part A

Project Title: Studies of Child Growth, Development and Behavior, and Disease Patterns in Primitive Cultures

Sub-Project I: Study of the developmental patterning of the human nervous system (A cybernetics of human development)

3. Investigation of non-recurrent phenomena (objectives and selectivity to be used in documentation of aperiodic phenomena to preserve masimum information).

Principal Investigators: E. Richard Sorenson and D. Carleton Gajdusek, M.D.

Project Description:

Although the problem of nonrecurring phenomena is usually left to the historian, knowledge of past non-repeatable occurrences is of crucial inportance to studies like ours which must draw heavily upon data from changing and disappearing life situations where return to the undifferentiated data provided by the original situation is needed to gain insights and clues to further inquiry. Further return to the data of the original phenomena is required to review them in the light of the insights subsequently gained.

Continuing attention has thus been given to the problem of disappearing phenomena associated with child development, and the previously published method of dealing with the investigation of nonrecurring phenomena is being refined, particularly as regards procedures aimed at the reduction of field editing of the original phenomena and the use of multiple recording devices.

Part B included: Yes

Part B: Publications

Sorenson, E.R. and Gajdusek, D.C.: The investigation of nonrecurring phenomena. The research cinema film. Nature, 200: 112-112, 1963.

Sorenson, E.R. and Gajdusek, D.C.: The study of child behavior and development in primitive cultures. A research archive for ethnopediatric film investigations of styles in the patterning of the nervous system. II. History of scientific motion pictures. Pediatrics, in press.

Part A

Project Title: Studies of Child Growth, Development, and Behavior, and Disease Patterns in Primitive Cultures

Sub Project II: Human Evolutionary Study in Isolated Primitive Groups

1. Kuru.

A. Epidemiology of Kuru.

Principal Investigators: D. C. Gajdusek, M.D. and M. P. Alpers, M.D.

Other Investigators: P. W. Brown, M.D., E. Richard Sorenson, and Patricia Hunt.

Cooperating Investigators: Dr. Vincent Zigas, Department of Public Health, New Guinea; Mr. Jack Baker, Taipini, New Guinea; Dr. and Mrs. Robert Glasse, Okapa, New Guinea; Mrs. Lucy Hamilton Reid, Lae, New Guinea; Dr. Richard Hornabrook, Kuru Research, Okapa, New Guinea; Department of District Administration, New Guinea, and Department of Public Health, New Guinea.

Project Description:

Since the disease kuru was first discovered in late 1956 a file of all known cases has been kept by this office and represents the most complete record available of kuru. During this year the records in this file were vigorously checked against internal error and inconsistency and against all other available records.

This file comprises some 1450 cases. The records were analyzed by age and sex in two three-year periods, an early (1957-1959) and a late (1961-1963), and the results compared. The comparison was made by sex and age distribution and mortality rates for all cases, then successively by linguistic group, tribal group, and village. Mortality figures for the villages were plotted geographically and possible groupings related to kuru mortality, not covered by traditional tribal groups, looked for. Sex ratio and child:adult ratio of cases were calculated for each of these breakdown levels (total, linguistic group, tribal group and village) within each period and the periods compared. The data were then analyzed by single year from 1957 through 1963 for age and sex distribution and mortality rates, for each

of the breakdown levels, and the figures for each year plotted against time. At the tribal group and village level correlations between mortality rate and the sex and age ratios were looked for.

In addition, population data for the same periods were analyzed for population growth, death rate, birth rate, natural increase, infant mortality, proportion of childbearing females, and sex ratio of the total population and various age groups for each available census, and attempts were made to construct age specific tables for kuru mortality from the limited data available.

The times of onset and death in each case where they were given were carefully reassessed and a study of the seasonal onset of kuru was made. The duration of the disease was calculated for every case where information was available and the relationship between age and duration was studied and compared in each of the two three-year periods.

The principal conclusion--that of the striking disappearance of kuru among the younger age groups in recent years--proved surprising and had not previously been appreciated. The work of keeping up the records file from widely different sources continues, and it will be possible to make analyses quickly and readily from time to time to see if the trends apparent at present are continued into the future.

In addition to these extensive studies of the whole kuru region, intensive dietary, social and environmental studies were carried out in three widely-separated villages in the region as part of the search for environmental factors which may be involved in the etiology of kuru.

Part B included: Yes

Part B: Publications

Alpers, M. and Gajdusek, D.C.: Changing patterns of Kuru: Epidemiological changes in the period of increasing contact of the Fore people with Western civilization. American Journal of Tropical Medicine and Hygiene, September 1965. (in press)

Alpers, M.: Epidemiological changes in Kuru, 1957 to 1965. In: Slow, Latent and Temperate Virus Infections, Monograph - Editors: D. C. Gajdusek and C. J. Gibbs, Jr., NINDB, NIH, Bethesda, Maryland, 1965. (in press)

Reid, Lucy H. and Gajdusek, D.C.: Studies on kuru. VII. A nutritional evaluation of the Moke Fore, 1957-1958. American Journal of Tropical Medicine and Hygiene. (in press)

Part A

Project Title: Studies of Child Growth, Development, and Behavior, and Disease Patterns in Primitive Cultures

Sub Project II: Human Evolutionary Study in Isolated Primitive Groups

1. Kuru.

B. Clinical and Epidemiological Laboratory Studies on Kuru.

Principal Investigators: D. C. Gajdusek, M.D. and M. P. Alpers, M.D.

Other Investigators: C. J. Gibbs, Ph.D., P. W. Brown, M.D., Patricia Hunt and Joanne Samuelson, Mint Basnight.

Cooperating Investigators: Dr. Vincent Zigas, Department of Public Health, New Guinea; Dr. A. V. G. Price, Department of Public Health, New Guinea; Dr. Richard Hornabrook, Kuru Research, Okapa, New Guinea; Dr. Charles Wisseman and Dr. Donald Helbig, Department of Microbiology, University of Maryland, Baltimore; Dr. Frank Schofield, Department of Public Health, New Guinea; Dr. Harry Feldman, State University of New York, Syracuse, New York; Dr. Irving Kagan, Dr. Elliott Milgram and Dr. Kenneth Walls, Parasitology Laboratory, Communicable Disease Center, Atlanta, Georgia; Dr. Robert Chanock, National Institute of Allergy and Infectious Disease, NIH; Dr. William Pond, University of Miami School of Medicine, Miami, Florida; Dr. Rodney Jung, Tulane University, Medical School, New Orleans, Louisiana; Dr. Philip Paterson, New York University School of Medicine, New York; Dr. Leon Rosen, Pacific Research Station, Hawaii, and others.

Project Description:

In conjunction with clinical and epidemiological studies on kuru patients and the population in which the disease occurs, specimens of blood, urine, cerebro spinal fluid, feces, hair, nails and post mortem tissue have been obtained from kuru victims and kuru-free controls in the kuru region and neighboring populations. In addition, articles of diet, clothing, salt,

soil and cooking materials have been collected for study. Over the years these have been subjected to biochemical analysis for the usual routine clinical pathology and more intensive search for trace metal deficiencies and accummulations. Serology against a large number of viral, bacterial, parasitic and fungal agents has been or is being carried out.

In general, the results of this extensive study have proved negative as far as providing a lead towards the etiology of kuru. They have, however, provided a great deal of information on the ecology and sero epidemiology of the kuru region. Further work still has to be done in each of these many small studies to increase the number in the samples tested and to check the critical studies by newer or different techniques. This work of correlation and completion of the laboratory studies on kuru has formed an important project for this year. Even when the major initial studies have been concluded, the search for environmental factors which may operate in kuru will continue; the established serum bank from cases going back to 1957 will enable new tests and techniques to be applied immediately to a large sample of cases.

Inoculation experiments in the laboratory searching for a transmissible agent in kuru are reported in (NDB (CF)-62 OAD 969-I).

Part B included: Yes

Part B: Publications

Gajdusek, D.C., Zigas, V, Price, A.V.G., and Alpers, M.: Studies on kuru. VIII. Laboratory studies on kuru. (in preparat ion)

Gajdusek, D.C., Zigas, V., Price, A.V.G., and Alpers, M.: Studies on kuru. IX. Antibody patterns in kuru patients and in residents of the kuru region and in surrounding populations. An introduction to a collaborative study. (In preparation)

Serial No. NDB (CF) OAD 65-1282 (II-1)

Part A

Project Title: Studies of Child Growth, Development, and Behavior, and Disease Patterns in Primitive Cultures

Sub Project II: Human Evolutionary Study in Isolated Primitive Groups

1. Kuru

C. Human Genetic Factors in the Kuru Region.

Principal Investigators: D. C. Gajdusek, M.D. and M. P. Alpers, M.D.

Other Investigators: P. W. Brown, M.D., Patricia Hunt, Joanne Samuelson, and Vicky Bigish, Mint Basnight.

Cooperating Investigators: Dr. Roy Simmons, Commonwealth Serum Laboratories, Melbourne, Australia; Dr. Cyril Curtain, Baker Medical Research Institute, Melbourne, Australia; Dr. Vincent Zigas, Department of Public Health, New Guinea; Dr. Richard Hornabrook, Kuru Research, Okapa, New Guinea; Dr. Alexander Bearn, Rockefeller Institute, New York; Dr. Arthur Steinberg, Department of Biology, Western Reserve University, Cleveland, Ohio; and Dr. Baruch Blumberg, Institute of Cancer Research, Philadelphia, Pa.

Project Description:

The pattern of red blood cell and serum genetic factors and a study of the serum proteins have been reported for kuru patients and controls from the kuru region. This study has now been amplified to include a larger sample of cases from within the region and extended to cover most of the linguistic groups of the Eastern Highlands. The number of genetic factors looked for has been increased to include further red cell blood groups, Gs and Gm serum groups, haptoglobins, hemoglobins, transferrins, and pseudocholinesterase and the presence of glucose-6-phosphate dehydrogenase and other enzyme deficiencies.

Part B Included: Yes

Part B: Publications

Simmons, R.T., Gajdusek, D.C. and Alpers, M.: Blood Group Genetic Patterns in Population Isolates in and Adjacent to the Kuru Region, Eastern Highlands, New Guinea. (in preparation)

Gajdusek, D.C., Alpers, M, Curtain, C., and Steinberg, A.G.: Human Genetics of Population Isolates in and Adjacent to the Kuru Region, Eastern Highlands, New Guinea. (in preparation)

Part A

Project Title: Studies of Child Growth, Development, and Behavior, and Disease Patterns in Primitive Cultures

Sub Project II: Human Evolutionary Study in Isolated Primitive Groups

1. Kuru

D. Kuru Research Films and Special Films on Kuru.

Principal Investigators: D. C. Gajdusek, M.D., E. Richard Sorenson, and M. P. Alpers, M.D.

Other Investigators: Judith Shaw and Dr. Teresita Elizan.

Project Description:

The raw footage taken in the field has been documented, titled, and edited into research film form with accompanying narration. From this research film file made for a wider purpose reported in (NDB (CF) OAD 65-1282 (I)), those portions showing kuru patients are being extracted for full length kuru research films. These are then being edited to make a number of smaller special films illustrating certain features of kuru.

Part B included: No

Part A

Project Title: Studies of Child Growth, Development, and Behavior, and Disease Patterns in Primitive Cultures

Sub Project II: Human Evolutionary Study in Isolated Primitive Groups

2. Motor neuron disease and other degenerative diseases in New Guinea and in other inbred Pacific Island populations.

Principal Investigator: D. C. Gajdusek, M.D.

Other Investigators: M. Alpers, M.D., E. Richard Sorenson, and Mint Basnight.

Cooperating Investigators: Dr. Jan ten Brink, Vught, Netherlands; Dr. Ken Dresser, Pirimapoen, West New Guinea; Dr. Jonathan Hancock, Lae, New Guinea; Dr. Richard Hornabrook, Kuru Research, Okapa, New Guinea; Dr. Frank Schofield, Maprik, New Guinea; Dr. R. L. Kirk, W.H.O., Geneva, Switzerland; Dr. Jean Guiart, Musee des Arts Africains et Oceaneans, Paris, France, and Department of Public Health, New Guinea.

a. Motor neuron disease in the Jaqai and Awju regions of West New Guinea.

b. CNS defects associated with endemic goiterous cretinism in the Highlands of West New Guinea.

c. Tremor syndromes in New Guinea.

d. Familial periodic paralysis in Tongariki, New Hebrides.

e. Post encephalitic syndromes in New Guinea natives.

Part B included: No

Part A

Project Title: Studies of Child Growth, Development, and Behavior, and Disease Patterns in Primitive Cultures

Sub Project III: Studies of Isolated Micronesian Populations

2. Response to live measles virus vaccine in immunologically virgin populations without circulating measles virus (with special attention to response in susceptible adults and pregnant women and their offspring).

Principal Investigators: Paul W. Brown, M.D., D. Carleton Gajdusek, M.D.

Other Investigators: Mint Basnight, Nancy Rogers

Cooperating Investigators: Dr. Daniel J. Schneider, Assistant Director, Medical Services, U.S.P.T.T., Saipan

Project Description:

Objectives: 1) Investigation of immune response in immunologically virgin populations to live attenuated measles virus vaccine; and 2) evaluation of safety of fetus in women immunized during pregnancy.

Methods Employed: Measles antibody determinations by hemagglutination-inhibition test as previously described and tissue culture neutralization tests.

Patient Material: Island natives of the Yap District for current phase of project; and from isolated Micronesian and Melanesian populations for future work.

Major Findings: and

Significance: Demonstration of 1) satisfactory HAI and neutralizing antibody rises in over 99 per cent of the immunized population one year after immunization, and 2) a trend toward lower titers in older age groups, and in subjects in any age group having the mildest clinical reactions.

Course: Continued follow-up of measles antibody levels over the next few years and continuing assessment of children born to women immunized during pregnancy.

Part B included: Yes

Part B: Publications

Brown, P., Basnight, M. and Gajdusek, D.C.: Measles immunization of susceptible island populations in Micronesia. Am. J. Epid. (in press).

Part A

Project Title: Studies of Child Growth, Development, and Behavior, and Disease Patterns in Primitive Cultures

Sub-Project III: Studies of Isolated Micronesian Populations

3. Influenza A_2: a virgin-soil epidemics (epidemiological, clinical, and immunological response and discovery of populations without previous experience with Type A or Type B influenza).

Principal Investigators: Paul W. Brown, M.D., D. Carleton Gajdusek, M.D.

Other Investigator: J. Anthony Morris, Ph.D.

Cooperating Investigators: Dr. M.F. Veki, District Director of Health,, Dr. A. Bolbuu, Yap Hospital, Yap District

Project Description:

Objectives: 1) Epidemiological, clinical, and immunological evaluation of an epidemic of A_2 influenza in populations without previous exposure to influenza, and 2) evaluation of selected influenza vaccines in other still susceptible populations with little or no previous exposure to influenza.

Methods Employed: Tissue culture neutralization tests.

Patient Material: Island natives of the Yap District of Micronesia.

Major Findings: Severe morbidity and a mortality of nearly seven per cent in the most isolated populations. Significant antigenic overlap between the infecting contemporary A_2 strain and earlier A_2 strains. Some antigenic overlap with A1 and A subtypes, mostly at low neutralizing titer levels. Suggestion of booster response in individuals previously infected with A1 or A influenza virus. Demonstration of almost 1400 people with a similar pattern of susceptibility on other atolls, to Type A and Type B influenza.

Significance and Course: Documentation of the lack of previous experience with influenza, as a critical factor in the consequent severity of infection by the virus. Demonstration of low level antigenic overlap between A_2, A1 and A influenza subtype viruses with A_2 infection capable of eliminating an immune response to A1 or A strains. Conginuing investigation of influenza baccines in ummunologically virgin-populations.

Part B: Yes

Part B: Publications

Brown, Paul, Gajdusek, D.C. and Morris, J.A.: Epidemic A_2 influenza in isolated Pacific atoll populations free from influenza for 25 years, and the discovery of other still unexposed populations. In preparation.

Gajdusek, D.C., Brown, P. and Morris, J.A.: Influenza A_2 epidemic in isolated Pacific atoll populations with no previous experience with prototype or contemporary A_2 or contemporary B strains. Program and Abstracts of the Soc. Pediat. Res., 35th Annual Mtg., May 4-6, Seattle; p. 92, 1965.

Part A

Project Title: Studies of Child Growth, Development, and Behavior, and Disease Patterns in Primitive Cultures.

Sub-Project IX: Experimental Developmental Neuro-pediatrics of Infantile Programming: an Empirical Approach to the Languages of Information Input into the Nervous System

Principal Investigators: D. Carleton Gajdusek, M.D. and Michael Nicholson

Other Investigators: Judith Shaw

Student Assistants: Vicky Bigish

Project Description:

Objectives: 1) To discover, isolate and define a behavioral characteristic which is grossly influenced by infantile experience; 2) to study in great detail the input information producing the behavioral change and treat the material as digitals of information theory.

Methods Employed: A survey was used to seek out and identify a suitable behavioral characteristic and the type of infantile experience required to produce observable change. The survey examined three species of experimental animals, seven discrete abstract behavioral actions, three gross behavioral patterns and five classes of infantile treatment at four levels each. A complete cinemagraphic time-lapse analysis was made with full statistical treatment.

The problems of raising mice alone with only a mother and forced weaning with isolation proved to be difficult to overcome, but these techniques are now in use. The selected behavioral test (a total behavioral pattern) has evolved to a well defined simple three-level test scored independently by several people.

Major Findings: The survey resulted in the identification of the least variable mouse strain and the selection of a class of infantile experiences producing dramatic behavioral changes. The phenomenon of producing an animal with great fear of attack by simply varying the early social experiences is more definite than we had hoped, and the elucidation of the precise input requirements producing this reaction is intensively under way.

Experiments are now being designed involving total social deprivation (including mother) and extreme deprivation of individual senses from birth. The information gained from these experiments will be used to create an original and dramatic example of infantile programming, to be used in attempts to analysis of the "language of sensory input" into the developing nervous system of the young mouse.

Serial No. NDB (CF)-62 OAD 969(I-IV)
1. Collaborative-Field Research
2. Office of Associate Director
3. Bethesda, Maryland
Projects NDB(CF)-62 OAD 971
and NDB(CF)-63 OAD 1140
have now been integrated
into this project.

PHS-NIH
Project Report
July1, 1964 through June 30, 1965

Part A

Project Title: Slow, Latent, and Temperate Virus Infections of the Central Nervous System of Man and Animals

Sub-Project I: Attempts to isolate transmissible agents from subacute and chronic diseases of the nervous system.

Sub-Project II: Workshop-symposium on slow, latent, and temperate virus infections.

Sub-Project III: Fluorescent antibody technique in localizing neurotropic virus antigen in whole animals.

Sub-Project IV: Studies on the ecology, epidemiology and pathogenesis of arbovirus infections.

Principal Investigators: D. Carleton Gajdusek, M.D. and Clarence J. Gibbs, Jr., Ph. D.

Other Investigators: Paul W. Brown, M.D., Michael Alpers, M.D., Nancy G. Rogers, Mint Basnight

Technical Assistants: Michael Sulima, Helene Gilbert, Edward van Steinberg, Alfred Bacote, Michael Nicholson

Student Assistants: John Francis, Kenneth Bethea

Man Years (computed for 12 month period)

Total:	8.25
Professional:	2.25
Other:	6.00

Most of the studies reported in this section are being conducted in the NINDB laboratory of Slow, Latent, and Temperate Virus Infections at the Patuxent Research Center, Laurel, Maryland, in collaboration with the Bureau of Wildlife and Sports Fisheries, U.S. Department of Interior. Additional associated facilities are with the Laboratory of the Study of Child Growth and Development and Disease Patterns in Primitive Cultures, of which this project is a part and from which it originates (see Project Report Serial No. NDB(CF)65-OAD 1282 (I-IX)).

Part A

Project Title: Slow, Latent, and Temperate Virus Infections of the Central Nervous System of Man and Animals

Sub-Project I: Attempts to isolate transmissible agents from subacute and chronic diseases of the nervous system.

Principal Investigators: Clarence J. Gibbs, Jr., Ph.D., D. Carleton Gajdusek, M.D.

Other Investigators: Michael Alpers, M.D., Paul W. Brown, M.D., Nancy Rogers

Cooperating Investigators: David Poskanzer, M.D., Massachusetts General Hospital, Boston; Richard T. Johnson, M.D., Department of Neuropathology, Cleveland Metropolitan Hospital, Cleveland; J. Craighead, M.D., Peter Bent Brigham Hospital, Boston; L.V. Zilber, M.D., Gamalaya Institute, Moscow, U.S.S.R.; Leonard T. Kurland, M.D., Mayo Clinic, Rochester, Minnesota; John Sever, M.D., NINDB; J.A. Morris, Ph.D. and H. Hopps, M.S., DBS; B. Dessel, M.D., Veterans Hospital, Wood, Wisconsin; A.J. Kenyon, D.V.M., Univ. Connecticut, Storrs; Mrs. E. Beck and Prof. P.M. Daniel, Department of Neuropathology, Maudsley Hospital, London, England; P.A. Palsson, D.V.M., M. Gudnadottir, M.D. and H. Thormar, Ph.D., Institute of Experimental Pathology, Keldur, Iceland; C.M. Eklund, M.D. and W. Hadlow, D.V.M., Rocky Mountain Laboratory, NIAID, Hamilton, Montana; J. Hourrigan, R. Reisinger and H. McDaniel, Animal Research Section, U.S. Department of Agriculture, Washington; E. Dustman and C. Herman, Patuxent Research Center, Department of Interior.

Project Description:

Objectives: In general, the basic objectives established at the initiation of this program remain the same: 1) to attempt to demonstrate infectious etiology for progressive degenerative diseases of the nervous system of man and animals by isolation techniques; 2) to determine the epidemiological significance of established strains of slow, latent and temperate viruses in infections of the nervous system of man and animals; 3) to determine the biological, physical and chemical characteristics of known slow viruses, in order to define the basic biology of this new group of infectious and pathogenic agents; 4) to develop new techniques for the successful isolation, identification, and characterization of the etiological

agents of progressive degenerative diseases of the nervous system of man and animals; 5) to develop suitable serological tests for early detection and identification of agents involved in these neurological diseases; and 6) to develop techniques for the establishment of cell cultures of autopsied and biopsied tissues from the central nervous system of humans and animals affected with degenerative diseases of the nervous system, and to apply these techniques to the study of masking, latency, and temperateness of viruses that cause slow but progressive and fatal diseases of the nervous system.

Methods Employed: Although the concept of "slow infections" of viral etiology has been known for over a decade, and a number of viruses capable of inducing progressive degenerative diseases of the nervous system of animals following long term incubation of many months to several years are now known to exist (scrapie, visna, maedi, Aleutian mink disease, mink encephalopathy), it is only relatively recent that detailed studies into the nature of the physical, chemical, and biological characteristics of these viruses have been undertaken in an effort to develop techniques to be employed in our efforts to elicit an infectious etiology for the chronic, progressively degenerative diseases of the nervous system of man. The etiological significance of these animal viruses in chronic diseases of the nervous system of man has not, to date, been explored. During the period covered by this report experiments have been continued in an effort to determine the etiology and pathogenesis of neurological diseases such as kuru (9 cases), amyotrophic lateral sclerosis-Parkinsonism dementia syndrome of Guamanians (10 cases), amyotrophic lateral sclerosis of both the familial and sporadic types observed in humans residing in the continental U.S. (9 cases), the Zilber monkey-adapted amyotrophic lateral sclerosis agent from the U.S.S.R., Dawson's inclusion body encephalitis (1 case), and thymus tissue from a case of myosthenia gravis in a human. New experiments were initiated by the inoculation of several species of primates (chimpanzees, rhesus, cynomolgus and African green monkeys), sheep, small laboratory animals, embryonated hen's eggs, and a variety of primary and stable cell cultures with surgically biopsied and freshly autopsied central nervous system tissue from patients diagnosed as affected with multiple sclerosis (3 cases), Schilder's disease (1 case), Parkinsonism (1 case), multifocal leucoencephalopathy (1 case), metachromatic leucoencephalopathy (1 case), subacute sclerosing leucoencephalopathy (1 case), sporadic amyotrophic lateral sclerosis (1 case), and Parkinsonism-dementia (1 case). In addition, similar isolation experiments were initiated on surgically biopsied tissue from a patient suspected of being affected with Aleutian mink disease. Many of the methods employed in these studies have been and are being developed during the course of our studies on scrapie virus of sheep.

During the period covered by this report we have determined the size of the scrapie agent by filtration and density gradient experiments; demonstra-

ted the thermostability of the agent, its sensitivity to organic solvents, enlarged and intensified our efforts to develop an antigen-antibody system in scrapie infections, experimentally induced an encephalopathy in mice by inoculation of serum and CNS tissue of sheep naturally infected with scrapie. As part of these studies we have been developing procedures to be followed in the cultivation of viable cells in vitro from surgically biopsied CNS tissue, thymus tissue of human origin, and posterior dorsal root ganglia from newborn rhesus monkeys; in addition, we have been studying the recovery and cultivation of viable cells from liquid nitrogen preserved central nervous system tissue obtained at autopsy from kuru patients in New Guinea.

All inoculated animals, eggs, and cell cultures are closely observed for infection and/or neurological complications at which time tissue will be taken for serial passage into experimental hosts and for neuropathological studies. All inocula are thoroughly studied for other microroganisms that may be present, e.g. bacteria, rickettsia, PPLO, fungi, parasites, etc.

Major Findings: Animal inoculation with human materials: The earliest experiments on the inoculation of primates with CNS tissue from a human affected with kuru was initiated in January 1963. This was followed by inoculation of primates, small laboratory animals, and cell cultures in August and September 1963, with specimens from an additional seven human cases of kuru, as well as five cases of amyotrophic lateral sclerosis and ALS-Parkinsonism-dementia. In the ensuing months, as mentioned under Methods Employed additional specimens from humans affected with kuru, ALS, ALS-PD, as well as other neurological diseases, were inoculated into primates and other animals. To date, in these extremely long term studies, no evidence of disease attributable to the inocula has been observed; although several of the inoculated primates were lost in the early post-inoculation period, their deaths were directly attributable to heavy parasitism, diarrhea and other 'non-specific' causes.

Primary cell cultures of human and animal origin: Employing standard tissue culture techniques we have successfully cultivated central nervous system tissues in vitro from human kuru specimens, as well as from freshly biopsied CNS tissue specimens from patients with multiple sclerosis and multifocal leucoencephalopathy; a thymus cell line has been established from a patient with myosthenia gravis. These in vitro studies are being closely followed for determination of antigenic fractions that may react with known specific viral antisera as well as for electron microscopy for detection of viral-like intracellular particles. In order to establish potentially susceptible cell lines we have successfully cultivated CNS and thymus tissue from normal and scrapie infected mice and posterior doraal root ganglia from newborn rhesus monkeys. Cell line susceptibility studies to known viruses are underway.

Scrapie: To date, efforts to develop a method to use in the detection of antibody to scrapie virus have been unsuccessful; these have included

experiments to elicit neutralizing antibody, complement fixation, hemagglutination inhibition and precipitating antibody and fluorescent antibody by the direct and indirect techniques. In contrast to these negative results, we have determined the size of the scrapie agent to be in the range of 27 - 43 , and demonstrated the agent's slight sensitivity to diethyl ether as compared to the fully susceptible arboviruses and the fully resistant enteroviruses. Further, in addition to experimentally inducing an encephalopathy in mice by inoculation of CNS tissue from eight naturally occurring cases of scrapie in sheet, we have been able to demonstrate, for the first time, scrapie-like disease in mice injected with serum from a scrapie infected ram. The rather unorthodox nature of the scrapie agent is manifest in its resistance to heat when compared to most known viruses, e.g. infectious particles capable of inducing encephalopathy in mice were recovered following exposure to a temperature of 100°C for 15 minutes, although 99 % of the viral infectivity had been destroyed following exposure to the temperature for as short as two minutes. These studies strongly suggest a structure of double stranded DNA for the scrapie agent.

Significance to Bio-medical research and the Program of the Institute: These long term studies represent the first major effort to experimentally induce certain neurological diseases of humans in experimental animals and to isolate their causative agents. They formed the major foundation for the convening during this year of a highly successful symposium on slow, latent and temperate virus infections at the National Institutes of Health with participants from several of the states, as well as from foreign countries. These meetings have resulted in a monograph, now in press, that is expected to serve as the only current available reference on diseases of this nature. Further, from the detailed physical, chemical and biological characterization of known slow viruses, such as scrapie, we are developing techniques for the study of degenerative diseases of man, not only in our own laboratory, but throughout the world.

Proposed course of the project: 1) Continued long term observation of inoculated animals. Continued serial studies on the fractionation of serum specimens from these animals for the determination of shifts in the elcecrophoretic patterns, as well as their antibody status which may be indicative of sub-clinical infections; 2) continued effort to develop suitable antigen-antibody systems for the study of established strains of 'slow'viruses; application of these new techniques to the study of human disease; 3) intensification of the development and application of fluorescent-antibody techniques with the model virus and other chronic viruses, such as LCM and rabies, which may remain latent for many years before clinically apparent disease becomes manifest; 4) greater emphasis on growth, cultivation and establishment of cell culture lines of "target-organ" nervous tissue from human and animals with degenerative diseases of the nervous system, as well as from cases of "auto-immune" diseases in an effort to isolate an etiological agent in a controlled in vitro environment, detection of abnormal antigenic fractions giving indirect evidence of disease and possible association with known viruses and establishment of new cell lines for the study of viral

growth, maturation, and measurement of interferon or interferon-like substances; 5) increased efforts to adapt strains of "slow" viruses to growth, serial propagation and characterization in tissue and cell culture systems; 6) continued efforts toward the development of procedures for the successful isolation of etiological agents responsible for degenerative diseases of the CNS, such procedures to include cell culture blocking techniques, detection of endo symbiotic relationship of masked, latent, or temperate viruses and cells in culture and chemotherapeutic lowering of animal resistance to infection.

Part B included: Yes

Part B: Publications

Gibbs, C.J., Jr., Gajdusek, D.C. and Morris, J.A.: Further observations in mice following inoculation of scrapie goat and sheep brain material. Publication of Symposium on Scrapie, U.S. Department of Agriculture and National Academy of Sciences, National Research Council, 1964.

Gajdusek, D.C. and Gibbs, C.J., Jr.: Attempts to demonstrate a transmissible agent in kuru, amyotrophic lateral sclerosis and other subacute chronic nervous system degenerations of man. Nature, 204: 257-259, 1964.

Neumann, Meta A. and Gajdusek, D.C.: Neuropathologic findings in exotic neurologic disorders among natives of the Highlands in New Guinea. J. Neuropath. Exp. Neurol., 23: 189, 1964.

Neumann, Meta, Gajdusek, D.C. and Zigas, V.: Neuropathologic findings in exotic neurologic disorders among natives of the Highlands of New Guinea. J. Neuropath. Exp. Neurol., 23: 486-507, 1964.

Alpers, M. and Gajdusek, D.C.: Changing patterns of kuru: epidemiological changes in the period of increasing contact of the Fore people with Western civilization. Am. J. Trop. Med. Hyg., in press.

Beck, Elisabeth, Daniel, P.M. and Gajdusek, D.C.: A comparison between the neuropathological changes in kuru and in scrapie, a system degeneration. Proc. Vth Inter. Cong. of Neuropath., in press. (To be presented at the Vth Inter. Cong. Neuropath., Zurich, Aug. 30-Sept. 3, 1965)

Morris, J.A., Gajdusek, D.C. and Gibbs, C.J., Jr.: Spread of scrapie from inoculated to uninoculated mice. Proc. Soc. Exper. Biol. Med., in press.

Gibbs, C.J., Jr. and Gajdusek, D.C.: General considerations of slow virus infections. Proc. Inter. Sympos. Rabies, in press (Presented at the Inter. Sympos. Rabies, Geneva, May 30-31, 1965).

Part B: continued

Gibbs, C.J., Jr., Gajdusek, D.C. and Morris, J.A.: Viral characteristics of the scrapie agent in mice. In: Slow, Latent and Temperate Virus Infections, D.C. Gajdusek and C.J. Gibbs, Jr., editors; NINDB, National Institutes of Health, U.S. Government Printing Office, Washington, D.C., 1965.
(Monograph based on the Workshop and Symposium on Slow, Latent and Temperate Virus Infections, National Institutes of Health, December 7-9, 1964)

Serial No. NDB (CF)-62 OAD 969 (LL)
New Project 1964

Part A

Project Title: Slow, Latent, and Temperate Virus Infections of the Central Nervous System of Man and Animals

Sub-Project II: Workshop-Symposium on Slow, Latent and Temperate Virus Infections.

Principal Investigators: D. Carleton Gajdusek, M.D. and Clarence J. Gibbs, Jr., Ph.D.

Other Investigators: Michael Alpers, M.D., Paul W. Brown, M.D.

Cooperating Investigators: See: List of Participants and Table of Contents in: Slow, Latent and Temperate Virus Infections. Editors: D. Carleton Gajdusek and Clarence J. Gibbs, Jr., National Institutes of Health, 1965 (Monograph of papers presented at the Workshop-Symposium)

Part B included: Yes

Part B: Publications

Gajdusek, D.C. and C.J. Gibbs, Jr., editors: Slow, Latent and Temperate Virus Infections, NINDB, National Institutes of Health, U.S. Government Printing Office, Washington, D.C.
(Monograph on Workshop and Symposium held at the National Institutes of Health, Bethesda, Maryland, December 7-9, 1964; 44 papers, 7 definitive bibliographies)

Contributions in above Monograph from this section:

Gajdusek, D.C.: Kuru in New Guinea and the origin of the NINDB study of slow, latent and temperate virus infections of the nervous system of man.

Gibbs, C.J., Jr. and Gajdusek, D.C.: Attempt to demonstrate a transmissible agent in kuru, amyotrophic lateral sclerosis, and other subacute and chronic progressive nervous system degenerations of man.

Alpers, M.: Epidemiological changes in kuru, 1957 to 1963.

Gibbs, C.J., Jr., Gajdusek, D.C. and Morris, J.A.: Viral characteristics of the scrapie agent in mice.

Morris, J.A., Gajdusek, D.C. and Gibbs, C.J., Jr.: Spread of scrapie from inoculated to uninoculated mice.

Definitive Bibliographies:
Kuru in New Guinea
Scrapie with References on Rida
Visna
Maedi
Aleutian Mink Disease with References on Chediak-Higashi Syndrome
Chediak-Higashi Syndrome
Hemorrhagic Fevers in South America

Serial No. NDB (CF)-62 OAD 969 (III)
Continued Project 1963 Serial No.
NDB (CF)-63-OAD 1140

Part A

Project Title: Slow, Latent and Temperate Virus Infections of the Central Nervous System of Man and Animals

Sub-Project III: Fluorescent antibody technique in localizing neurotropic virus antigen in whole animals

Principal Investigators: Paul W. Brown, M.D., D. Carleton Gajdusek, M.D.

Other Investigators: Michael Alpers, M.D., Nancy Rogers, Clarence J. Gibbs, Jr., Ph.D. and Mint Basnight

Cooperating Investigators: J. Hotchin, M.D., Division of Laboratories, New York Department of Health, Albany

Project Description:

Objectives: To use lymphocytic choreomeningitis as a model in establishing the methodology to be used in studies of other central nervous system diseases wherein a suspicion of possible virus etiology is entertained and to define the pathogenesis of LCM. To prepare fluorescent animal antisera to antigens in brain tissue of kuru, amyotrophic lateral sclerosis and other chronic central nervous system degenerative disorders, for use as reagents.

Methods Employed: Lymphocytic choreomeningitis in mice is used as a developmental tool in establishing fluorescent antibody techniques in the localization of virus antigen during a slow virus infection involving the central nervous system.

Significance and Course: Study of LCM as an example of the slow or latent virus infection and, particularly, as an example of the phenomenon of acquired immune tolerance to infection in the mouse. This work on the pathogenesis of lymphocytic choreomeningitis is used both as a valuable addition to our knowledge in its own right and to establish proceedings which may aid in localizing virus antigen if any is present in other chronic central nervous system diseases wherein virus etiology is suspected. Diseases in which we will use the techniques are kuru, ALS, scrapie and Aleutian mink disease. Satisfactory preparation and staining of cross-sections of whole mice has been developed. The course and distribution of LCM has been defined in neonatally infected primary tolerant immune mice sacrificed at intervals after infection. The distribution of virus has also been defined in primary

tolerant immune mice afflicted with 'late disease' occurring eight to 12 months after infection. Hyperimmune sera are being prepared to human brain material from cases of chronic and subacute central nervous system disease..

Part B included: Yes

Part B: Publications

Publications other than abstracts from this project:

Brown, Paul: Fluorescent antibody studies on the pathogenesis of lymphocytic choriomeningitis in neonatally infected primary tolerant immune mice. In preparation.

Hotchin, J. and Brown, Paul: Late disease of lymphocytic choriomeningitis infected primary tolerant immune mice. In preparation.

Serial No. NDB (CF)-62 OAD 969-IV
Continued Project 1962 Serial No.
NDB(CF)-62 OAD 971

Part A

Project Title: Slow, Latent, and Temperate Virus Infections of The Central Nervous System of Man and Animals.

Sub-Project IV: Studies on the Ecology, Epidemiology, and, Pathogenesis of Arbovirus Infections.

1. Epidemic Hemorrhagic Fever in Calcutta.
2. Seroepidemiology of Arbovirus Infections in Ecologically Isolated Primitive Indigenous Populations:
   a) Seroepidemiology of Alaskan Populations
   b) Seroepidemiology of the populations of the Carribian and Central and South American Countries with particular reference to Puerto Rico, Bolivia and Paraguay
   c) Seroepidemiology of Australasian populations

Principal Investigators: Clarence J. Gibbs, Jr., Ph.D.
D. Carleton Gajdusek, M.D.

Cooperating Investigators: K. Shah, M.D. and F. Bang, M.D., School of Public Health, Johns Hopkins University; B. K. Aikert, M.D., Institute of Post Graduate Medical Research, Calcutta; J. Casals, M.D., Yale University School of Medicine; J. Brody, M.D., Arctic Health Research Center, Anchorage, Alaska; R. Randall, D.V.M., WRAIR, WRAMC; J. Sever, M.D., NINDB Perinatal Research Branch, NIH; C. Wisseman, M.D., E. B. Helwig, M.D. and E. Rosenzweig, Ph.D., Department of Microbiology, University of M ryland School of Medicine; T. Work, M.D., CDC, Atlanta; F. Schofield, M.D., Director Medical Research, Territory Papua and New Guinea; R. Hornabrook, Kuru Research Center, Okapa, New Guinea; P. Allen, Ph.D. V and R Division, Ft. Detrick, Maryland; R. Taylor, M.D., School of Public Health, University of California, Berkley, California.

Part A.

1. Epidemic Hemorrhagic Fever in Calcutta

Principal Investigators: Clarence J. Gibbs, Jr., Ph.D.
Keerti Shah, M.D.
B. K. Aikert, M.D.
D. Carleton Gajdusek, M.D.

Cooperating Investigators: F. Bang, M.D., J. Casals, M.D., R. Randall, D.V.M.

Objectives: To further define the immunogenic and antigenic Characteristics of the strains of Chikungunya virus isolated from humans during an epidemic of acute febrile illnesses.

Methods Employed: Three strains of identical or closely related group A arboviruses isolated from acute phase serum specimens from humans with a febrile illness characterized by hemorrhagic manifestations were studied by complement-fixation, hemagglutination-inhibition and neutralization tests conducted in vitro and in the latter case in vivo. The immunological and antigenic relationship of these three strains of Chikungunya were compared to prototype strains of this group A virus isolated from humans and animals in widely separated geographical areas of the world. Human sera, collected in India several months and years before the current epidemic, were tested for antibodies to Chikungunya virus.

Major Findings: Three strains of virus isolated from acutely ill humans during an outbreak of febrile illness with hemorrhagic manifestations in Calcutta, India, were identified as strains of Chikungunya virus belonging to Casals' group A arboviruses. In routine complement-fixation, hemagglutination-inhibition, in vivo and in vitro neutralization tests and plaque inhibition tests, it was not possible to separate the strains one from another or from prototype strains of Chikungunya virus isolated from other geographical areas of the world. In contrast, when the kinetics of hemagglutination-inhibition was determined by more precise methods it was found that the Calcutta isolates were more closely related to strains of Chikungunya isolated in Bangkok, Thailand, than to those strains of the virus isolated in Africa. Antibodies

to these newly isolated Indian strains of the virus were detected in the sera collected from humans in the Calcutta area many months to years prior to the outbreak of the epidemic, indicating prior existence of this virus in the community rather than a recent introduction.

Part B included: Yes

Part B: Publications

Shah, K.V., Gibbs, C.J., Jr. and Banerjee, G.: Virological investigation of the epidemic of haemorrhagic fever in Calcutta: isolation of three strains of Chikungunya virus. Indian Journal Med. Res., 52: 676-683, 1964.

Shah, K.V., Gilotra, S.K., Gibbs, C.J., Jr. and Rozeboom, L.E.: Laboratory studies of transmission of Chikungunya virus by mosquitoes: a preliminary report. Indian J. Med. Res., 52: 703-709, 1964.

Gajdusek, D.C.: Epidemic hemorrhagic fever with renal syndrome. Discussion of the paper by M.P. Chumakov. Proc. 7th Int. Cong. Trop. Med. Malaria, 3: 292-295, 1964.

Gajdusek, D.C. and C.J. Gibbs, Jr.: Definitive bibliography, Hemorrhagic Fevers in South America. In: Slow, Latent and Temperate Virus Infections; Editors, Gajdusek, D.C. and Gibbs, C.J., Jr., NINDB, National Institutes of Health, U.S. Government Printing Office, 1965. (Monograph on Workshop and Symposium held at the National Institutes of Health, Bethesda, Maryland, December 7-9, 1964; 44 papers, 7 definitive bibliographies).

Part A.

2. Seroepidemiological Patterns of Arboviruses in Ecologically Isolated Primitive Indigenous Populations.

Principal Investigators: Clarence J. Gibbs, Jr., Ph.D.
D. Carleton Gajdusek, M.D.
Other Investigators: M. Alpers, M.D. and P. Brown, M.D.
Cooperating Investigators: J. Casals, M.D., J. Brody, M.D., C. Wisseman, M.D., E. B. Helwig, M.D., E. Rosenzweig, Ph.D., T. Work, M.D., F. Schofield, M.D., R. Hornabrook, M.D., P. Allen, Ph.D., R. Taylor, M.D., J. Sever, M.D.

Objectives: 1) To determine the seroepidemiological patterns of arboviruses in geographically remote areas of the world; 2) to determine the etiological significance of these viruses in human diseases in these areas; and, 3) to establish a baseline epidemiology for the early detection and recognition of newly introduced viruses.

Methods Employed: During the conduct of our field studies with primitive groups and through the efforts of collaborative investigators we have established a large serum file, kept largely at -20°C and partly at -70°C, of specimens from indiginous populations from many remote and isolated ecological regions of the world. These specimens have been used for many biochemical genetic and seroepidemiological studies among which arbovirus investigations receive prominent attention. These latter studies include tests for complement-fixing, hemagglutination-inhibition, precipitating, neutralizing and fluorescent antibodies to each of the several (18-20) serological groups of arboviruses. Results of these tests are biostatistically analyzed to determine pattern time of virus invasion into a population, age attack groups, and patterns of duration of immunity. An effort is made to continue surveillance in an effort to detect viruses capable of causing epidemics of fatal febrile illnesses in humans.

Major Findings:

a) Seroepidemiology of Alaskan populations: In collaboration with investigators at the Arctic Health Research Center, Anchorage, Alaska, over 500 human sera have been collected and are in the process of being tested against arboviruses of serological groups A, B, C, Bunyamwera and California. No patterns have, as yet, emerged from these studies though it can be reported that many

sera react with the antigens prepared from group B viruses.

b) Seroepidemiology of the populations of the Carribian and Central and South American Countries with Particular Reference to Puerto Rico, Bolivia and Paraguay. During the course of our studies sera that had been collected from the Guayaki indians of Paraguay were tested for HI antibodies to arboviruses. The results of these tests were totally unexpected in that not a single one of the sera reacted to a group B antigen. This is interpreted to mean that not only has the area never been invaded by any of the commonly detected Dengue viruses but just as significantly a population of jungle people exist that are highly susceptible should yellow fever virus become introduced into the area. Although the testing of these sera is not complete, preliminary results further indicate that group A antibodies will be relatively rare suggesting the area to be susceptible to members of this group e.g., Venezulean equine encephalomyelitis.

c) Seroepidemiology of Australasian populations. Data from several years of extensive seroepidemiological investigations for antibodies to arboviruses in several thousand serum specimens collected during our field studies on primitive populations in Australasia have been prepared by Dr. Helwig for computer analysis in the laboratory of Dr. Charles Wisseman, Department of Microbiology, University of Maryland School of Medicine, where these studies have largely been conducted under the supervision of Dr. E. Rosenzweig. These studies and their purpose were summarized in previous annual reports (NINDB-CFR-OAD-7). Publications summarizing the general project have appeared this year (Part B), and several papers dealing with detailed analysis of the ecological, ethnological and disease pattern data for restricted subregions of Australasia , with respect to the antibody patterns of group A, group B and group C viruses, in various age groups are now in preparation.

Other studies of restricted population groups have been done in collaboration with Dr. J. Sever , NINDB. These include a few arbovirus antibody analyses as well as testing for many other groups of virus antibodies.

In our Patuxent laboratory we have surveyed sera from several

Melanesian populations, particularly from the Eastern Highland region of New Guinea. In several instances we have detected foci of apparently specific antibodies to group A viruses particularly eastern equine encephalomyelitis (EEE) virus. Follow-up studies to determine the nature and extent of possible EEE disease in humans are now in progress.

A wider variety of arbovirus antigens have been employed in seroepidemiological tests on sera from Guam. To date, 62 sera collected from Chamorros on Guam, with diagnosed cases of familial type amyotrophic lateral sclerosis or Parkinsonism dementia, have been tested for hemagglutination-inhibition antibodies to group A and group B viruses. Forty-seven of the 62 serum specimens (75.8%) had HI antibodies to one or more of the six group B virus antigens employed in the test (Japanese B, Dengue 1, Dengue 2, St. Louis encephalitis, yellow fever and Powassan); only 2 of the 62 sera (3.2%) had antibody to the group A antigen tested (Chikungunya). The high prevalence of group B antibody is thought to be due to the epidemic some years ago of Japanese B virus and to the frequent occurrence of Dengue in the area.

Part B included: Yes

Part B: Publications

Gibbs, C.J., Jr., Bruckner, E.A., and Schenker, S.: A Case of Apeu Virus Infection. American Journal of Tropical Medicine and Hygiene, 13:1 (January), 109-113, 1964.

Wiebenga, N.H., Shelokov, A., Gibbs, C.J., Jr., and Mackenzie, R.B.: Epidemic Hemorrhagic Fever in Bolivia. II. Demonstration of Complement-Fixing Antibody in Patients' Sera with Junin Virus Antigen, American Journal of Tropical Medicine and Hygiene, 13:4 (July) 626-628, 1964.

Wisseman, C.L., Jr., Gajdusek, D.C., Schofield, F.D. and Rosenzweig, E.C.: Arthropod-borne virus infections of aborigines idigenous to Australasia. A preliminary report. Bulletin of the World Health Organization, 30:2, 211-219, 1964. Also in: Papua and New Guinea Medical Journal, March 1965.

Wisseman, C.L., Jr., Gajdusek, D.C., Rosenzweig, E. C., and Helbig, D.W.: Seroepidemiology of Australasia. I. Arvor Virus Antibodies in Australian Aborigines of Cape York Peninsula. (in preparation)

Helbig, D.W. Report on the Arbor Virus seroepidemiology of Australasia. Methodology of Data Processing. Report presented at the Communicable Disease Center, Atlanta, Georgia, May 1965.

Wisseman, C.L., Jr., Gajdusek, D.C., Brown, P.W., Rosenzweig, E.C., and Helbig, D.W.: Seroepidemiology of Australasia. II. Arbor Virus antibodies in the Micronesian population of the Western Caroline Islands. (in preparation)

Gibbs, C.J., Jr.: The Arboviruses. Paper presented at The Graduate School of Arts and Sciences, The Catholic University of America, Washington, D.C., February 1964.

Gibbs, Clarence J., Jr. Antigenic Classification of Arboviruses and their significance to human disease. Presented to U.S. Navy Research Unit, National Naval Medical Center, Bethesda, Maryland, March 1964.

ANNUAL REPORT
July 1, 1964--June 30, 1965
Biometrics Branch - Collaborative and Field Research
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

A. SCOPE OF PROGRAM

The activities of the Biometrics Branch during the period July 1, 1964--June 30, 1965 may be divided into the following general programs:

1. Model Reporting Area for Blindness Statistics (MRA).

2. Statistics on Severe Hearing Impairment and Deafness.

3. Research Projects with Other Investigators.

4. Statistical Consultation and/or Service Given to Investigators Outside of NINDB on Other Projects.

5. Statistical Consultation and/or Service to Clinical and Basic Research Investigators at NINDB in the Areas of Neurology and Blindness.

The status of each of the above programs is given below.

1. The Model Reporting Area for Blindness Statistics.

(a) Primary Objectives

The purposes of the Model Reporting Area are to make better statistics available on blind persons and to stimulate research in the field of blindness. To accomplish these objectives, the Model Reporting Area will encourage complete reporting of blind persons to the registers maintained by member State agencies; improve the records concerning the reported blind; use a common definition of blindness; standardize the recording of essential information; and disseminate statistics derived from the registers to all who are interested in the problems of blindness.

(b) Significant Accomplishments, July 1, 1964--June 30, 1965

Each of the States comprising the Model Reporting Area, which was organized in 1962, maintains a State-wide register of blind persons. On July 1, 1964 the Area was composed of ten such States, the State of Oregon having been added to the membership earlier in the year. However, as indicated below, a number of other States were actively engaged in

improving their registers so as to meet the requirements of the Model Reporting Area. This resulted in the addition of two such States to the Model Reporting Area, raising the total membership to 12 States by June 30, 1965.

All but two of the member States now have their registers on a tabulating type punch card system, but these two States have also expressed an interest in converting their present registers to such a system with the assistance of the Biometrics Branch. Accordingly, one of these States has initiated a request for financial and technical assistance under contract with the NINDB to effect the revision of its register. It is expected that the other State will request similar assistance in the near future. The use of punch cards throughout the Model Reporting Area provides great flexibility in the production of tabulations of blindness statistics derived from the program.

Acting upon request from various States, the Biometrics Branch provided technical and consultative assistance to a number of state agencies interested in membership in the Model Reporting Area. Seven States were under contract during the year to establish or improve their existing registers so as to meet the requirements of the Model Reporting Area. Upon satisfactory completion of its contract, two of the States were admitted to the program and membership is imminent for two other States. The other three States under contract should achieve membership during the next fiscal year. The 17 States which are members or actively working toward membership represent approximately one-third of the total United States population.

Tabulations on the incidence and prevalence of blindness and severe vision impairment were produced for the Model Reporting Area member States covering the calendar year 1963. These data were published as the 1963 Statistical Report of the Model Reporting Area, the second in a series of annual statistical reports. Included in the Report were tabulations on additions to the registers during the year (i.e. incidence), number registered at the end of the year (i.e. prevalence), and removals from the register. The tables were prepared according to such characteristics as sex, age, race, degree of vision, and cause of blindness. Also published during the year were two papers on reported incidence of blindness in the Model Reporting Area States for 1962 and 1963, including a paper on the causes of blindness.

The Model Reporting Area program is providing a means for wide-scale adoption of a Standard Classification of Causes of Severe Vision Impairment and Blindness, which has been developed and sponsored by the National Society for the

Prevention of Blindness. The Biometrics Branch has played an integral part in revising the Standard Classification and the Index to the Classification during the past year. Also, the Biometrics Branch sponsored a Workshop on the Coding of Causes of Blindness (see below). Through the Workshop and a quality control program, which was initiated immediately following the Workshop, the Biometrics Branch is attempting to achieve a high degree of uniformity in the coding of causes of blindness throughout the entire Model Reporting Area. In the quality control program the Biometrics Branch staff reviews the coding of causes of blindness of all or a random sample of all ophthalmological eye reports processed by the member States. The Model Reporting Area program has also aided in the adoption by a number of Model Reporting Area States of a standard eye report form prepared by the National Society for the Prevention of Blindness.

(c) Major Plans for Fiscal Year 1966

It is planned to continue providing technical and financial assistance and support to States interested in membership in the Model Reporting Area, until the program has achieved a significant representation of the United States population. An annual conference will be held if sufficient funds are included in the budget for this purpose. If not, it may be necessary to switch to a biannual conference. Consultation and guidance may be provided to agencies in other countries which are interested in the MRA program, utilizing, if possible, P.L. 480 funds.

The Model Reporting Area program continues to receive wide support throughout the country and abroad. In addition to previous endorsements received from the American Optometric Association and the Council of State Directors of Vocational Rehabilitation, the Model Reporting Area was endorsed by the American Academy of Ophthalmology and Otolaryngology and a similar endorsement was to be considered by the Section on Ophthalmology of the American Medical Association, at its Annual Convention in June 1965.

Great interest has been expressed by a number of foreign countries, such as Egypt, Israel, and India, in the development of Model Reporting Area procedures as a means of securing improved blindness prevalence and incidence statistics by their governments. In addition, the World Health Organization at Geneva, and the Royal Commonwealth Society for the Blind in London have expressed continuing interest in the development of the Model Reporting Area.

(d) Special Activities

A three-day Workshop was held to train coders in the use of the Index to the 1965 revision of the Standard Classification of Causes of Blindness and Severe Vision Impairment. It was attended by coders from each of the 17 States associated with the Model Reporting Area program.

The Fourth Annual Conference of the Model Reporting Area was held in Bethesda, Maryland on May 20 and 21, 1965. It was attended by about 70 persons, including representatives of the member States, non-Model Reporting Area States that are improving their registers for anticipated affiliation with the Model Reporting Area, various Federal and voluntary agencies, and other groups and individuals interested in the problem of blindness.

2. Statistics on Severe Hearing Impairment and Deafness.

(a) Primary Objectives

Adequate statistics on prevalence, incidence and causes of severe hearing impairment and deafness, as well as on characteristics of the deaf, are urgently needed in order to mount effective local, State, and national programs to prevent and control such impairments.

(b) Significant Accomplishments, July 1, 1964--June 30, 1965

At the present time no operation exists in this country to collect statistics on the incidence and prevalence of severe hearing impairment and deafness by cause, degree of loss, and other variables. That the lack of such data is a serious obstacle to adequate prevention and control activities is evident. This belief has also been expressed by medical and other authorities in the field. Any attempt to start collection of such data would be a significant accomplishment. A proposal for such collection was suggested at a Conference called by the Biometrics Branch in March 1964. The proposal, endorsed by the Conference was designed to investigate the possibilities of gathering data on the student deaf from schools and classes for the deaf. Despite the fact that the proposal was subsequently endorsed by the Conference of Executives of American Schools for the Deaf, American Speech and Hearing Association, American Hearing Society, Alexander Graham Bell Association, and the American Academy of Ophthalmology and Otolaryngology, the proposal has not been implemented due to a shortage in positions and funds. It should further be mentioned that the American Academy of Ophthalmology and Otolaryngology has, upon request of the Biometrics Branch, established a Subcommittee of the Committee on Conservation of Hearing to

develop a classification of causes of severe hearing impairment and deafness which would, of course, be essential in securing comparable cause statistics. Again, due to a shortage of funds, the Subcommittee has not been activated.

(c) Major Plans for Fiscal Year 1966

If several additional staff positions become available, it is hoped that a plan of data collection based on recommendations coming out of the above-mentioned Conference, may be instituted. Guidance will be sought from a Planning Group to be set up and from the Subcommittee mentioned above.

(d) Special Activities

None.

3. Research Projects with Other Investigators.

(a) Primary Objectives

The objective of this program is to conduct significant research in the area of neurological and sensory disorders of interest to the NINDB.

(b) Significant Accomplishments, July 1, 1964--June 30, 1965

(1) A study of association between factors of pregnancy, labor and delivery and the occurrence of blindness in children: the purpose of this retrospective study is to determine whether mothers of blind children are characterized by an excess of selected prenatal and perinatal disorders as compared to mothers of a control population of live births surviving the neonatal period. The study is being done with the cooperation of the New York State Commission for the Blind and the New York City and the New York State Departments of Health. Included in the study are approximately 600 blind children and a control group composed of a stratified sample of approximately 8,000 recorded live births during the study period in New York State. Data pertaining to conditions of pregnancy, labor and delivery, for both cases and controls, were obtained from the confidential medical supplements of the respective birth certificates. All of the necessary information has been abstracted, tabulations have been prepared, and the final analysis is in progress.

(2) A study of survivorship and causes of death among the blind: the objectives of the study, which has been completed, were (a) to determine survival and sight-

recovery rates for blind persons by age, sex, and causes of blindness; (b) to determine the probabilities of dying from major causes of death and the distribution of causes of death among blind persons compared to corresponding rates and distributions observed in the general population. This was a follow-up study of 11,732 persons first registered as legally blind in Massachusetts during the 20-year period, 1940-1959. Blind persons experienced poorer survivorship than expected at all ages up to 75 years, with little if any differences apparent at ages 75 years and over. Marked differences were noted for ages 25-64 years. Survival rates for persons with diabetes as the cause of blindness were extremely low in comparison to the general population. The causes of death among the blind especially showing large excesses in mortality over expected were diabetes and heart disease. Sight-restored rates were, in general, rather low. Throughout, findings were similar for males and females.

(3) A retrospective study of association of perinatal factors with strabismus in Negro children: the objectives are (a) to determine whether mothers of Negro children with strabismus are different from mothers of Negro control children in reporting prenatal and obstetric complications; and (b) to determine whether the distribution of birth weights for children with strabismus is different from that of children selected as controls. The records of various eye clinics in Baltimore have been used for selection of study cases. Birth certificates in the Baltimore City and the Maryland State Health Departments were used for selection of controls. Information on prenatal and obstetric complications was obtained from hospital records. The case group consists of some 400 children meeting specified criteria with regard to diagnosis of strabismus, age, place of birth, and other factors. An appropriately matched control group is being used for comparison. Data have been collected, abstracted, coded, and keypunched into IBM cards. Plans are under way for tabulating and analyzing results.

(4) Evaluation of the levels of ocular tension in an Italian-American population and comparison of tensions obtained by three methods: the purpose of the project, conducted jointly with the Epidemiology Branch, was to describe tension levels in an Italian-American community, Nesquehoning, Pennsylvania. Since a similar population in Roseto, Pennsylvania, had shown an unusually low frequency of deaths due to myocardial infarction, it was believed that a survey in the Nesquehoning area

would offer valuable information pertaining to the possible association between chronic vascular disease and chronic simple glaucoma. Three methods of tonometry were employed in examining each individual: applanation sitting, applanation supine and Schiotz. As the descriptive facet of the survey was deemed most important bias due to confounding change of position and the resultant time interval in the two applanation series could not be resolved. Preliminary investigation has disclosed no striking peculiarities in the population but significant differences among tonometric methods.

(5) Study of awareness, attitudes, and performance in reporting persons to blindness registers: this study is being done under contract with the American Foundation for the Blind. It is concerned with the actual reporting behavior of ophthalmologists, EENT specialists, and optometrists, as well as their awareness of, and attitudes regarding, the reporting of blind persons to registers. Also under study are the operations of State registration agencies, including the communications which have evolved between these agencies and the reporting sources with whom they deal. This study will supplement the work of the Model Reporting Area for Blindness Statistics (MRA) by focusing on the key problem of obtaining complete reporting of blind persons. After a number of pre-tests of drafts of questionnaires, final versions of questionnaires for directors of blindness registration agencies, their consulting ophthalmologists, and ophthalmologists and optometrists in the field were submitted for Budget Bureau clearance with the expectation that field interviewing would begin on or about May 1, 1965. All 28 States with mandatory blindness registers were to be included for study so that comparisons of reporting behavior and attitudes might be made for ophthalmologists and optometrists separately in States having mandatory reporting against those that do not; in MRA member States versus non-member States, by region of country; and by the amount and type of information provided (regarding registers) by the register agencies. In addition to the 28 register agency directors and the 28 consulting ophthalmologists to these agencies, it is planned to interview 500 ophthalmologists located in the 193 cities of the 28 States with 50,000 population or more (as of April 1, 1960) and 200 optometrists (located in the same cities). All interviews, to be conducted by trained interviewers of a commercial research firm under sub-contract, will be face-to-face, except for the State of Hawaii where a mail questionnaire will be used. Endorsements for the study were obtained from the American Academy of Ophthalmology and Otolaryngology and the American Optometric Association.

(6) American Public Health Association Monograph on Neurological and Sense Organ Disorders: this is one of the series of monographs, sponsored by the American Public Health Association, which includes members of the Biometrics Branch among the authors. This monograph will contain information on the mortality, morbidity, and epidemiology of neurological and sense organ disorders, including a review of pertinent literature already published. Detailed tabulations were prepared on mortality from various neurological disorders to be included in the monograph, and these will be incorporated, where pertinent, in the various chapters of the monograph. A meeting of the authors was held and plans for completion of the monograph were detailed.

(7) A study of association between factors of pregnancy, labor and delivery and the occurrence of deafness in children: the purpose of this retrospective study is to determine whether mothers of deaf children are characterized by an excess of selected prenatal and perinatal disorders as compared to mothers of the total population of live births surviving the neonatal period. The study is being done with the cooperation of the New York State Education Department, and the New York City Board of Education, and the New York State and New York City Departments of Health. Included in the study are approximately 900 deaf children and a control group composed of a stratified sample of approximately 8,000 recorded live births during the study period in New York State. Data pertaining to conditions of pregnancy, labor and delivery, for both cases and controls, were obtained from the confidential medical supplements of the respective birth certificates. All of the necessary information has been abstracted. It is planned to prepare punch cards and to start analysis in the near future.

(8) A study of blindness statistics in Egypt: the objectives of this study are (a) to ascertain the feasibility of developing a blindness register on a pilot study basis in an Egyptian community as a mechanism for producing statistics on blindness (including severe vision impairment) needed for prevention and control activities and (b) to determine to what extent modifications may be made in procedures developed by the Model Reporting Area for Blindness Statistics (MRA) in the United States that, when applied in Egypt, will still permit production of statistics comparable to those of MRA. By adapting the standards of the MRA to the collection of data on blindness in urban areas in,

and rural areas outside of, Alexandria, Egypt, it is hoped to determine prevalence and incidence rates of blindness that will be comparable to those derived from MRA data. The project has three phases. In Phase I a random sample of 2,000 households (consisting of about 10,000 persons) will be selected. This sample will consist of persons from urban and rural areas, roughly in proportion to their numbers in the study population. No substitution or replacement of households in the sample will be permitted. A listing of all households in the urban and rural areas will provide an up-to-date complete frame of households from which to sample. When possible, all members of households falling into the sample will be tested for central visual acuity, and peripheral field where such visual acuity is better than 6/60 in the better eye with best correction. Visual acuity will be obtained with the use of portable vision testers which ensures standard lighting and distance, as well as privacy, during such examinations. These vision testers have been modified so that they can be used equally well in homes with or without electricity and in homes using either 110-volts or 220-volts. The survey will also include refraction (without drops) and measurement of peripheral field vision by means of portable perimetry. Trained examiners will be used for all testing. All persons determined by the survey to be blind, according to definition, will be examined later by ophthalmologists. These physicians will not only confirm the visual acuity, refractive and perimetric data determined by the survey, but will also attempt to reach a diagnosis and, possibly, the underlying cause of the condition. Attempts will be made before the study is started and periodically throughout its duration to ensure uniformity in visual testing by examiners and in diagnosis by ophthalmologists. The purpose of Phase II will be to build up a blindness register by attempting to have all the residents of the study areas (approximately 250,000 persons) visit the health centers or the University Hospital voluntarily for a vision examination identical to that given in Phase I. Again, all persons picked up as blind will be referred for ophthalmological examination. In addition, ophthalmologists and all agencies, hospitals and clinics serving the blind will be canvassed to get information on their known blind and will be encouraged to report blind cases to the register on a continuing basis. The results of the involuntary screening of Phase I will be compared with those of the voluntary screening in Phase II in order to determine which demographic groups are under-represented among those reporting for examination. This will permit

better focusing of educational efforts to bring about completeness of voluntary screening. Phase III will be a repeat of Phase I with re-examination of persons in the original sample of 10,000 persons still resident in the study areas in order to determine new cases of blindness that have developed in the interim and all cases that have had their vision restored. Emphasis will be placed on standardization of procedures in the uniform collection of data and, thus, assure as far as possible the comparability of data between the MRA and the Egyptian pilot study. It is expected that this study will provide precise information to indicate the extent to which data collection procedures of a highly developed country may and should be modified so that, for purposes of statistical evaluation, they are usable in a less sophisticated environment.

(c) Major Plans for Fiscal Year 1966

Work will be continued on the projects described above and papers will be prepared on those projects which will have been completed during the year. Where it is possible to undertake new projects, within the limits of personnel and budget, this will be done.

(d) Special Activities

None.

4. Statistical Consultation and/or Service Given to Investigators Outside of NINDB on Other Projects.

(a) Primary Objectives

The purpose of this program is to provide guidance or service upon request, to various agencies concerned with programs of interest to the NINDB. It is hoped that such efforts would produce research of a sound nature, avoid duplication of efforts by various agencies, help to coordinate existing or conflicting programs and help to yield a uniform and meaningful approach to the problem areas concerned.

(b) Significant Accomplishments, July 1, 1964--June 30, 1965

The Chief of the Biometrics Branch serves on the following committees:

Biostatistics Special Review Panel, Division of Research Grants, NIH
Advisory Committee on Operational Research, National Society for the Prevention of Blindness

Steering Committee, Blinded War Veterans Project, American Foundation for the Blind
Ad Hoc Committee on Sensory Research and Development, American Foundation for the Blind and Massachusetts Institute of Technology
Sub-Committee on Visual Impairment, Rehabilitation Codes
Ad Hoc Committee on Mental Retardation Program Statistics, DHEW
Scientific Advisory Council, Myopia Research Foundation
Scientific Advisory Committee, Massachusetts Institute of Technology Sensory Aid Evaluation and Development Center

Consultation was given to a representative of the Division of Occupational Health, Bureau of States Services, PHS, concerning the procedures that might be followed in developing a Model Reporting Area for Occupational Health, making use of experience in developing the Model Reporting Area for Blindness Statistics.

Consultation was rendered to the Department of Epidemiology, Columbia University School of Public Health and Administrative Medicine, regarding the distribution of deaths due to Parkinsonism, late effects of acute infectious encephalitis, and paralysis agitans.

Consultation was given to a representative of the Columbia Lighthouse for the Blind on the incidence and prevalence of blindness for the age group 16 through 64 years inclusive in Model Reporting Area States in connection with a plan to set up a Rehabilitation Center for the blind on a demonstration program basis to serve the States of Delaware, Maryland, Virginia, West Virginia and the District of Columbia.

Consultation to, and statistical work with, was undertaken with the Biometrics Branch, National Heart Institute.

In cooperation with the Computation and Data Processing Branch, National Institutes of Health, and with the Laboratory of Neurochemistry, National Institute of Mental Health, work on the mathematics of sequencing proteins and RNA's was undertaken.

A Symposium at the AAAS meeting in Montreal, Canada on Statistical Methodology and Problems in Palynology was organized and conducted. At this meeting a paper was given on "Null Models and Their Significance in Interpreting Pollen Frequencies."

(c) Major Plans for Fiscal Year 1966

Continuation of these activities.

(d) Special Activities

None

5. Consultation and/or Service Furnished to Clinical and Basic Research Investigators at NINDB in the Areas of Neurology and Blindness.

(a) Primary Objectives

The purpose of this program is to provide consultative and computational services in the design, analysis, and evaluation of research and experiments conducted by the clinical and basic research investigators at the Institute.

(b) Significant Accomplishments, July 1, 1964--June 30, 1965

Various units of NINDB were provided with consultative and computational services in the design, analysis, and evaluation of their experiments. This includes mathematical assistance for development of theoretical models of biological and psychological behavior.

Below are indicated the units in NINDB that received aid from the Biometrics Branch from July 1, 1964 thru June 30, 1965.

(1) Office of the Director

Editorial work on a paper dealing with psychological tests.

(2) Medical Neurology Branch

Statistical assistance and consulting was rendered as follows:

a. Consultation and analysis of muscle velocity curves. Computer analyses including least squares fits of Hills equation.

b. Consultation on a muscular dystrophy project.

c. Binomial distribution of the nuclei in muscles.

d. Studies in glucose tests and muscular dystrophy and ALS.

(3) Surgical Neurology Branch

Consultation, assistance and programming was given for the following projects:

a. Computer program was developed for correlation analyses of test scores.

b. Assistance and consultation was given on model of 3-way ANOVA for a mixed model with unequal n's.

c. Completed analysis of numerous covariance and correlation matrices obtained from psychological tests.

d. Chi-squares analyses were done by computer for a study of "Behaviour after Temporal Lobectomy and Temporal Craniotomy," to be published in Neurology.

e. Preparation of code lists for, liaison with Computation and Data Processing Branch in processing assistance on, and preliminary analysis of data of electrode implant project.

f. Assistance with the planning of the large scale Severe Head Injury Collaborative Study including preparation of code lists, proposals for a feasibility study, the protocol, etc.

g. Programming Mathatron for p log p and factor weights.

h. Preparing model and instruction for Lawley's Maximum Likelihood Method for obtaining factor weights.

i. Instruction on the mathematics of analysis of variance and factor analysis.

(4) Ophthalmology Branch

Consulting and statistical assistance in analyses of data was given on the following projects:

a. H-800 correlation analyses of intraocular systolic, diastolic pressures.

b. Correlation, regression analysis and analysis of covariance on intraocular pressure. Intraocular pressure analyses using the pre-learned correlation sequence on the Mathatron.

c. A 3-way ANOVA on flow rates was completed.

d. Correlation, regression, t-tests on eye muscular contractions.

e. Paired t-tests on retinal responses and scotopic energy.

f. t-tests on ophthalmological threshold responses.

g. Correlation and regression analysis of intraocular pressure and % turnover under influence of various drug dosages.

(5) Epidemiology Branch

a. Consultation on a long-term study of the occurrence of amyotrophic lateral sclerosis and Parkinsonism dementia in Guam, including advice on methods of analysis of the data.

b. Consultation on a study of mortality from eye tumors (including specimens of such tumors) in the United States during the period 1959-1961, involving cooperation from several States, the National Vital Statistics Division of the National Center for Health Statistics, and the Armed Forces Institute of Pathology.

c. Consultation on study design of proposed surveys of selected populations examined tonometrically.

d. Consultation regarding age adjustment of rates of amyotrophic sclerosis in Guam and in a Japanese city with radically different age distributions of the population.

(6) Laboratory of Neuropathology

a. Correlations, regressions, equal frequency ellipsis for data on nuclear and cytoplasmic size.

b. Frequency of infestation of parasites in monkeys.

(7) Laboratory of Neuroanatomical Sciences

a. Computer analysis of spike heights vs. mm. from crush for from 1 to 27 days after operation on dogs has been prepared.

b. Standard regression and correlation analyses.

c. Least squares fits of exponential curves using computer.

d. Computation of paired t-tests for preliminary experiment on cholinesterase production.

e. Regression and regression intercept analyses by computer to measure background activity of cholinesterase as well as the relation of sole plates to cholinesterase production.

f. Lens volume differences in an implantation study, t-tests.

g. Lens volume differences in chick embryo.

h. Cell population determinations in spinal cord of fish.

(8) Collaborative and Field Research

a. Computation of maximum likelihood of gene frequencies.

(9) Electroencephalography Branch

a. Refereed paper on Bayes theorem.

b. Statistical assistance on paper dealing with the dorsal column nuclei in the cat.

c. Statistical assistance and analysis in study of field-size data.

d. Preparation of a model for the programming of computation of blood group gene frequencies by the maximum likelihood method.

(10) NINDB

a. Course of instruction and notes on the Mathatron presented to 15 members of NINDB staff.

(c) Major Plans for Fiscal Year 1966

Continuation of the provision of consultative and computational services to clinical and basic research investigators at the Institute.

(d) Special Activities

None.

B. TIME DISTRIBUTION OF BRANCH ACTIVITY

Below are indicated data representing Biometrics Branch man-years of work for the 12-month period, July 1, 1964--June 30, 1965 for all activities and for each major project.

Biometrics Branch, NINDB
Man-Years of Work

All Activities

Total:	17 13/20
Professional	8 11/20
Other	9 1/10

Model Reporting Area for Blindness Statistics

Total:	4 7/10
Professional	3 9/10
Other	8/10

Statistics on Severe Hearing Impairment and Deafness

Total:	1/20
Professional	1/20
Other	—

Perinatal Study of Blindness in Children

Total:	3/5
Professional	9/20
Other	3/20

Survivorship Study

Total:	2 1/20
Professional	2/5
Other	1 13/20

Strabismus Study

Total:	7/20
Professional	1/20
Other	3/10

Italian-American Tonometry Survey

Total:	3/10
Professional	1/5
Other	1/10

Man-Years of Work

Study of Awareness, Attitudes and Performance in Reporting Persons to Blindness Registers

Total:	1/10
Professional	1/10
Other	—

United States Mortality from Neurological and Sense Organ Diseases: A.P.H.A. Monograph Series

Total:	3/20
Professional	1/20
Other	1/10

Perinatal Study of Deafness in Children

Total:	1/4
Professional	1/5
Other	1/20

Egyptian Blindness Study

Total:	1/5
Professional	1/5
Other	—

Mathematical Statistical Consultation

Total:	3 13/20
Professional	1 13/20
Other	2

Miscellaneous

Total:	1 1/2
Professional	11/20
Other	19/20

Administration

Total:	3 3/4
Professional	3/4
Other	3

C. PUBLICATIONS

Papers published by members of the Biometrics Branch since the last annual report:

Biometrics Branch: Annual Tabulations of the Model Reporting Area for Blindness Statistics: 1962 Statistical Report, PHS Publication No. 1176, Washington, D. C., 1964.

Biometrics Branch: Proceedings Conference on the Collection of Statistics of Severe Hearing Impairments and Deafness in the United States, 1964. PHS Publication No. 1227, Washington, D. C., 1964.

Biometrics Branch: Proceedings Third Annual Conference of the Model Reporting Area for Blindness Statistics, 1964, PHS Publication No. 1295, Washington, D. C., 1965.

Biometrics Branch: Annual Tabulations of the Model Reporting Area for Blindness Statistics: 1963 Statistical Report, PHS Publication No.1312, Washington, D. C., 1965.

Goldstein, H.: So That All May See. New Outlook for the Blind, 1964, 58, 280-284.

Goldstein, H.: Blindness Register as a Research Tool. Proceedings Symposium on Research in Blindness and Severe Visual Impairment, American Foundation for the Blind, 1964.

Goldstein, H.; Goldberg, I.D.; Rogot, E.; and Moorhead, H.B.: Reported Incidence of Blindness by Cause in 1963. Sight-Saving Review, 1964, 34, 214-233.

Goldstein, H.; Goldberg, I.D.; Frazier, T.M.; and Davis, G.E.; Cigarette Smoking and Prematurity, Publ. Health Reports, 1964, 79, 553-560.

Goldstein, H.; and Schein, J.D.: First Steps Toward the Collection of Uniform Statistics of Severe Hearing Impairment and Deafness in the United States. Amer. Ann. Deaf, 1964, 109, 400-409.

Gurian, J.; Cornfield, J.; and Mosimann, J.E.: Comparisons of Power for Some Exact Multinomial Significance Tests. Psychometrika, 1964, 29, 409-419.

Henderson, M.; Goldstein, H.; Rogot, E.; Goldberg, I.D.; and Entwisle, G.: Perinatal Factors Associated with Epilepsy in Negro Children. Publ. Health Reports, 1964, 79, 501-509.

Martin, Paul S.; and Mosimann, J.E.: Geochronology of Pluvial Lake Cochise, Southern Arizona. III. Pollen Statistics and Pleistocene Metastability. Amer. J. Science, 1965, 263, 313-358.

Merrill, C.R.; Mosimann, J.E.; Bradley, D.F.; and Shapiro, M.B.: Reconstruction of Protein and Nucleic Acid Sequences. II. Isotomers. Biophysical and Biochemical Review Communications, 1965, 19, 255-260.

Mosimann, J.E.: Statistical Methods for the Pollen Analyst: Multinomial and Negative Multinomial Techniques. In Handbook on Paleontological Techniques, edited by B. Kummel and D. Raup, Part III, 636-673, San Francisco, Cal., W. H. Freeman & Co., 1965.

Ommaya, A.K.; and Sadowsky, D.: A System of Coding Medical Data for Punched-Card Machine Retrieval. Epilepsia, 1964, 5, 192-200.

Rogot, E.; Goldstein, H.; and Goldberg, I.D.: Reported Incidence of Blindness in Selected States in 1962. Publ. Health Reports, 1964, 79, 785-788.

D. PROBLEMS ENCOUNTERED

In the last Annual Report of the Biometrics Branch, that is for the period ending June 30, 1964, the fact was mentioned that the Branch had suffered a loss of approximately 30% of its positions by administrative order over the period 1/1/63-6/30/64. There was further mention that unless several of the eight positions removed were restored to the Branch it might not be possible to meet the requests of States for assistance in improvement of blindness registers much less undertake exploration of problems involved in gathering data on the young deaf from schools and classes for the deaf. During the period covered by the present Annual Report, 7/1/64--6/30/65, no positions were restored. In addition, the Branch budget suffered a loss of approximately $25,000 as compared to the budget of the year before.

It is most evident that the functions of the Branch have been seriously impaired by the loss of positions and funds. It should be mentioned that the proposal for gathering data on the young deaf, mentioned above, has been endorsed by the American Academy of Ophthalmology and Otolaryngology, American Hearing Society, American Speech and Hearing Association, Alexander Graham Bell Association and the Conference of Executives of American Schools for the Deaf. However, this proposed program cannot be started until necessary positions and funds are made available to the Branch.

That the Biometrics Branch has, despite the above losses, been able to meet its commitments to the MRA, speaks highly of the determination and efficiency of Branch staff. However, it has been found necessary to curtail to an appreciable extent the field trips to MRA States that are an integral part of securing uniform data. The reduction in Branch funds available for contracts with States seeking to establish or to improve blindness registers will hinder to some extent the further development of MRA.

It has become evident that promotional opportunities for professional staff at the higher levels are non-existent in the foreseeable future. Not only will this make it difficult to keep present staff but will, no doubt, make it almost impossible to recruit new staff.

E. PROPOSED OBJECTIVES

To date, the energies of the Biometrics Branch have been directed, within the limits of staff and budget, to the aspects of the programs mentioned above. The Branch has and will continue to work for improved statistics on the blind as well as to make the statistics from State to State more comparable by continuing its present program of furnishing help to States in developing and improving their blindness registers in order to meet the standards for admission to the Model Reporting Area. Where necessary and possible, contracts with State register agencies for the blind will be undertaken in order to assist such agencies that could not otherwise meet the Model Reporting Area standards. The Biometrics Branch will attempt to stimulate States to utilize their blindness registers with greater effectiveness for administrative and research uses. Finally, within the limits of personnel and budget, it will attempt to initiate new research studies with good objectives and good study designs in the area of neurologic and sense organ disorders, in addition to carrying out those on which it is presently engaged. As hitherto, the Branch will continue to furnish statistical consultation to serve clinical and basic research scientists at NINDB as well as to outside investigators.

Serial No. NDB (CF) - 61 B 833
Continued Project since 1961
1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964--June 30, 1965

Part A

Project Title: Model Reporting Area for Blindness Statistics

Principal Investigators: Hyman Goldstein and Irving D. Goldberg

Other Investigators: Geoffrey G. Dell'Osso, Candler M. Hawkins, Ronald L. Jacobson and Helen B. Moorhead

Cooperating Units: Connecticut Board of Education of the Blind
Kansas Department of Social Welfare
Louisiana Department of Public Welfare
Massachusetts Department of Education
New Hampshire Department of Health and Welfare
New Jersey Commission for the Blind
New Mexico Department of Public Welfare
North Carolina Commission for the Blind
Oregon Commission for the Blind
Rhode Island Department of Social Welfare
Vermont Department of Social Welfare
Virginia Commission for the Visually Handicapped

Man-Years of Work

Total:	4 7/10
Professional:	3 9/10
Other:	8/10

Project Description:

Objectives:

The Model Reporting Area for Blindness Statistics is a voluntary association of States which maintain registers of persons with serious visual impairment, and is organized under the sponsorship of the National Institute of Neurological Diseases and Blindness. (For convenience, the MRA defines the term blindness to include severe visual impairment as described below.) Each member State has agreed to uphold the standards for membership, and may continue in membership as long as it upholds them. Periodic evaluation of the performance of each member State will be made by the NINDB in order to insure maintenance of these standards.

The two basic objectives, to which each member State subscribes and agrees to uphold, are to make better statistics available on blind persons and to stimulate research in the field of blindness. In order to accomplish this, the MRA will:

1. Encourage complete reporting of the blind, so that the number registered may more nearly reflect the true prevalence of blindness, and so that additions to the registers may more nearly reflect the true incidence;
2. Seek to improve the records concerning the reported blind, so that the causes of blindness and the characteristics of the blind may more easily be studied;
3. Use a common definition of blindness and standardize recording of essential information, so that data from different States can be more meaningfully compared or pooled in an effort to arrive at national statistics on blindness.

Study Population:

Each member State accepts for statistical purposes the MRA definition of blindness, which includes severe visual impairment as follows: "Visual acuity of 20/200 or less in the better eye, with best correction; or visual acuity of more than 20/200 if the widest diameter of the field of vision subtends an angle no greater than 20 degrees." Individuals with a progressive eye condition which does not yet meet the MRA definition are excluded. As understood here, the term best correction does not include devices which cannot be used continuously or which improve visual acuity to more than 20/200 only by restricting the field of vision to 20 degrees or less.

Each member State will attempt to include on its register all residents who fall within the MRA definition of blindness. Such registrants will constitute its MRA Register. The State may also register other persons under a wider definition of blindness, but only if these persons can be easily distinguished for statistical purposes. The MRA Register will not be confined to persons who have applied for services; nor will there be any arbitrary exclusion of persons on the basis of age, race, or any other factor outside the criteria of the MRA definition of blindness.

Information to be Collected:

Each member State will attempt to collect all of the items of information listed below for each registrant. It will routinely make efforts to clarify ambiguous reports, and to obtain the data missing from incomplete reports. The required items must appear on the register card (or its equivalent) for ready access. These items are the essential minimum; however, States are encouraged to collect additional pertinent information.

(a) Date of addition to the MRA register
(b) Type of addition to the MRA register (i.e. whether first addition or readdition)
(c) County of residence (or its equivalent)
(d) Date of birth
(e) Sex
(f) Race (except where recording such information is contrary to law)
(g) Age at onset
(h) Date of eye examination
(i) Discipline of examiner
(j) Degree of vision
(k) Standard classification of causes of severe vision impairment and blindness
(l) Date of removal from MRA register
(m) Reason for removal from MRA register

Each member State will continually correct the essential items on its register cards in accordance with any reports received of reinterviews or reexaminations of registrants. It will immediately remove from the MRA Register any person known to have died, moved out of State, or recovered vision beyond the MRA definition of blindness. Furthermore, it will redetermine the residence and blindness status of every person on its MRA Register during an annual clearance. A registrant may be considered cleared if he has received any service for the blind or has otherwise been contacted since the beginning of the calendar year, provided that there is no evidence that his status has changed since then. However, intensive efforts must be made to contact all other registrants. Contact may be made by mail inquiry, telephone call, or personal visit to the registrant himself, his relatives or neighbors, his physician, or an institution in which he resides; a personal visit will be made, if feasible when other methods fail. These intensive efforts will be completed before the end of the calendar year, and registrants who have not been located will be removed from the MRA Register by that time. It may then be assumed that those persons who remain on the MRA Register at the end of the calendar year are still resident in the State and still fall within the MRA definition of blindness.

Analysis:

Each member State will prepare certain tabulations of data from its MRA Register or will prepare a duplicate deck of IBM cards for each calendar year, in accordance with the specifications of the NINDB, and will submit them to the NINDB by February 15 of the following year.

Data based on the 1963 annual tabulations have been compiled and have been published in the second report of MRA tabulations from the 10 States that comprised the MRA in 1963. The major findings were as follows:

(a) There were 4,412 first additions and 84 readditions to the MRA registers in 1963. The rate per 100,000 population for all additions to the MRA registers was 15.6, ranging by State from a low of 9.0 per 100,000 in New Jersey to a high of 25.2 per 100,000 in Vermont.

(b) The total number of persons on the MRA registers at the end of 1963 was 43,362, or a rate of 150.7 per 100,000 population.

(c) The rates for additions generally remained low under under 45 years of age and increased only slightly with age up to age 45. Thereafter the rates increased markedly with age in each sex. Although the rates among the aged were somewhat higher for females, there did not appear to be any major sex differences in the rates for additions.

(d) Persons reported with vision of exactly 20/200 represented 27.2 percent of the MRA total. This high proportion probably indicates a lack of precise visual acuity measurement or lack of careful recording by the eye examiner, particularly because this level of acuity is sufficient to distinguish the "legally" blind. Some six percent of all additions were totally blind, and almost seven percent had vision greater than 20/200 but with visual field restricted to 20 degrees or less. An additional 11 percent of all additions had vision limited to either light perception or light projection.

(e) For all additions the three most frequent causes of blindness were principally senile cataract, diabetic retinopathy, and primary glaucoma.

Significance of Research:

Data on prevalence, incidence, visual acuity, and cause of blindness available through the MRA represent the only data of its kind, with uniform definition of blindness, for a population of this magnitude (i.e. about 15% of the United States population in 1963). As the MRA grows and becomes more representative of the country geographically and demographically it may become possible to project estimates of incidence and prevalence for the country as a whole with respect to age, sex, race, visual acuity, and cause of blindness. Such estimates are urgently needed for planning programs of prevention and control.

Serial No. NDB (CF) - 61 B 833

Proposed Course of Project:

In view of the fact that a State commits itself to maintenance of its blindness register along the standards of the MRA, once it is admitted as a member, the project will continue for an indefinite period of time with little cost to NINDB. Additional qualified States will be added to the MRA until adequate representation of the United States is achieved, within the resources of the Branch.

Part B included yes.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Biometrics Branch: Annual Tabulations of the Model Reporting Area for Blindness Statistics: 1962 Statistical Report, PHS Publication No. 1176, Washington, D. C., 1964.

Biometrics Branch: Proceedings Third Annual Conference of the Model Reporting Area for Blindness Statistics, 1964, PHS Publication No. 1295, Washington, D. C., 1965.

Biometrics Branch: Annual Tabulations of the Model Reporting Area for Blindness Statistics: 1963 Statistical Report, PHS Publication No. 1312, Washington, D. C., 1965.

Goldstein, H.: Blindness Register as a Research Tool. Proceedings Symposium on Research in Blindness and Severe Visual Impairment, American Foundation for the Blind, 1964.

Goldstein, H.; Goldberg, I.D.; Rogot, E.; and Moorhead, H.B.: Reported Incidence of Blindness by Cause in 1963. Sight-Saving Review, 1964, 34, 214-233.

Rogot, E.; Goldstein, H.; and Goldberg, I.D.: Reported Incidence of Blindness in Selected States in 1962. Publ. Health Reports, 1964, 79, 785-788.

Honors and Awards relating to this project: None

Serial No. NDB (CF) - 64 B 1185
New Project
1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964--June 30, 1965

Part A

Project Title: Statistics on Severe Hearing Impairments and Deafness.

Principal Investigator: Hyman Goldstein

Other Investigator: Irving D. Goldberg

Man-Years of Work

Total:	1/20
Professional:	1/20
Other:	—

Project Description:

Objectives:

The collection of adequate statistics on deafness and severe hearing impairments would be of great importance in arriving at national estimates of incidence and prevalence. These data are needed in administrative planning on a local, State, and national level for measures designed to prevent and control such impairments, and on planning specified types of research projects.

Study Population:

The collection of data on severe hearing impairments and deafness has not as yet, been instituted by the Biometrics Branch due to loss of several positions resulting from an economy drive. However, a Biometrics Branch proposal for the collection of data on students in schools and classes for the deaf was unanimously endorsed at a recent Conference on the Collection of Statistics on Severe Hearing Impairments and Deafness called by the Biometrics Branch, NINDB, and held in Bethesda, Maryland, March 19-20, 1964. This proposal was also endorsed unanimously at the 36th Regular Meeting of the Conference of Executives of American Schools for the Deaf, Riverside, California on April 16, 1964. The study population

of such a proposal would be the students at public and private residential and day schools as well as day classes for the deaf. Additional endorsements of the proposed project have been received from the American Speech and Hearing Association, American Hearing Society, Alexander Graham Bell Association, and the American Academy of Ophthalmology and Otolaryngology.

Information to be Collected:

Agreement would be reached by a Planning Group, including representatives of the schools and classes, on an operational definition of deafness, on standardization of data relating to degree of loss and cause of impairment, as well as other items of demographic and social importance to be included for uniform collection of data.

Significance of Research:

Collection of data, as indicated above, would furnish to the nation the first otologically and audiologically validated data on a large segment of the deaf population. Since such collection would be on a continuing basis, it would furnish continuing information on incidence and prevalence by various demographic and other variables. It would encourage various research studies dealing with uniformly collected data, on relationships between the impairment and characteristics of the deaf, and would, in effect, be a universe for the derivation of specific types of samples.

Proposed Course of Project:

Until the Biometrics Branch has positions restored to its budget to permit preliminary surveys of several schools for the deaf and the setting up of standards to be recommended to a Planning Group, little, if anything, can be done to implement this project.

Part B included yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Biometrics Branch: Proceedings Conference on the Collection of Statistics of Severe Hearing Impairments and Deafness in the United States, 1964. PHS Publication No. 1227, Washington, D.C., 1964.

Goldstein H.; and Schein, J.D.: First Steps Toward the Collection of Uniform Statistics of Severe Hearing Impairment and Deafness in the United States. Amer. Ann. Deaf, 1964, 109, 400-409.

Honors and Awards relating to this project: None

Serial No. NDB (CF) - 62 B 965
Continued Project since 1962
1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964--June 30, 1965

Part A

Project Title: Study of Association between Factors of Pregnancy, Labor, and Delivery and the Occurrence of Blindness in Children

Principal Investigator: Irving D. Goldberg

Other Investigators: Hyman Goldstein and Eugene Rogot

Cooperating Units: New York State Commission for the Blind
New York State Health Department
New York City Health Department

Man-Years of Work

Total:	3/5
Professional:	9/20
Other:	3/20

Project Description:

Objectives:

To determine whether mothers of blind children are characterized by an excess of selected prenatal and perinatal disorders as compared to mothers of the total population of live births surviving the neonatal period.

Study Population:

All children (about 600 cases) who meet the following five criteria:

1. Born during the period 1948-1960;
2. Classified blind by the New York State Commission for the Blind during the period 1948-1960;
3. Having blindness presumed to be due to unspecified prenatal or genetic factors or to otherwise unknown factors as indicated in the records of the Commission;
4. With birth certificate on file at the New York City or the New York State Department of Health;
5. Single-born.

Control Group:

A stratified sample of 2 in every 1,000 recorded live births in New York during the years 1948-1960 (some 8,000 controls in all).

Information Collected:

(a) Cases and controls: items appearing on birth certificates, viz. date and county of birth, sex of child, weight at birth, race of mother, age of mother, number of previous children, complications of pregnancy, complications of labor, operative procedure, Rh factor, presence or absence of congenital abnormality or birth injury.

(b) Cases only: cause of blindness (site/type and etiology), year classified blind.

Analysis:

Comparison between cases and controls on factors under (a) above. Insofar as possible, different categories (type/site) of blindness will be studied separately.

Major Findings:

Data still in process of analysis.

Significance of Study:

This is, to the knowledge of investigators, the first attempt to relate a wide range of prenatal and perinatal disorders and the occurrence of blindness in children. If such association is found this may furnish information that would lead to improvement in prevention of certain types of blindness in children.

Proposed Course of Project:

To finish analysis and interpretation of data and publish report.

Part B included no.

Serial No. NDB (CF) - 62 B 966
Continued Project since 1962
1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964--June 30, 1965

Part A

Project Title: Study of Survivorship and Causes of Death among the Blind

Principal Investigator: Eugene Rogot

Other Investigators: Hyman Goldstein, Irving D. Goldberg

Cooperating Units: Massachusetts Division of the Blind
Massachusetts Vital Statistics Office

Man-Years of Work

Total:	2 1/20
Professional:	2/5
Other:	1 13/20

Project Description:

Objectives:

To determine survival rates for blind persons by age, sex and cause of blindness and compare with corresponding rates observed in the general population.

To determine the distribution of causes of death for deaths among blind persons by age, sex and cause of blindness and compare with corresponding distributions observed in the general population.

Study Population:

All persons first registered blind by the Massachusetts Division of the Blind between January 1, 1940 and December 31, 1959. The closing date for followup was December 31, 1961. This provided 11,732 blind persons of all ages who were observed for differing periods of time up to 22 years from the date registered as blind by the Massachusetts Division of the Blind. Approximately 5,700 deaths occurred in the study group between January 1, 1940 and December 31, 1961. The definition of

blindness used is the legal definition in Massachusetts, namely, corrected vision of 20/200 or less in the better eye or peripheral field of vision contracted to 20 degrees diameter or less regardless of visual acuity.

Control Group:

The general population in Massachusetts was used as the control group. Survival rates have been computed based on the 1949-1951 life table for Massachusetts. The distribution of deaths by underlying cause of death have been obtained for Massachusetts for the study period from published data.

Information Collected on Cases:

Includes: date of birth, sex, race, date of registration, visual acuity, cause of blindness (by site and by etiology), date and reason for removal, underlying cause of death.

Analysis:

Life table analysis of cases compared with "expected" survivorship of general Massachusetts population and comparisons of cause of death distributions of cases and controls. Cases were studied by cause of blindness.

Major Findings:

Blind persons experienced poorer survivorship than expected at all ages up to 75, with little if any difference apparent at ages 75 and over. Marked differences were noted for ages 25-64. Survival rates for persons with diabetes as the cause of blindness were extremely low in comparison to the general population.

Significance of Study:

To the knowledge of the investigators this was the first attempt to study survivorship and causes of death among all known blind in a broad general population group (a study more than 30 years ago was limited to totally blind industrial policyholders in a life insurance company). The findings indicate which types of blindness result in higher risk of death from specific causes than in the general population, and may lead to prolonged survivorship through improved specific care and prevention programs.

Proposed Course of Project:

To publish report and pursue new leads for further studies.

Part B included no

Serial No. NDB (CF) - 63 B 1134
Continued Project since 1963
1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964--June 30, 1965

Part A

Project Title: Study of Perinatal Factors Associated with Strabismus in Children

Principal Investigator: Hyman Goldstein

Other Investigators: Candler M. Hawkins, Irving D. Goldberg, Eugene Rogot; Maureen Henderson, George Entwisle, University of Maryland School of Medicine

Cooperating Units: Wilmer Eye Clinic of Johns Hopkins Hospital, Baltimore, Maryland
Presbyterian Hospital, Baltimore, Maryland
Baltimore City Eye-Ear-Nose-and-Throat Hospital, Baltimore, Maryland
Baltimore City Health Department, Baltimore, Maryland
Maryland State Health Department, Baltimore, Maryland

Man-Years of Work

Total:	7/20
Professional:	1/20
Other:	3/10

Project Description:

Objectives:

To determine whether mothers of Negro children with strabismus are different from mothers of Negro control children in reported prenatal and obstetric complications.

To determine whether the distribution of birth weights of children with strabismus is different from that of children selected as controls.

Study Population:

The case study group (400 children) will meet the following criteria:

1. They must have had a diagnosis of strabismus made at either the Wilmer Eye Clinic of Johns Hopkins Hospital, Presbyterian Hospital Eye Clinic, or Baltimore City Eye-Ear-Nose-and-Throat Hospital in Baltimore. Strabismus is defined as a constant extra ocular muscle imbalance characterized by esotropia or exotropia (cases of esophoria and exophoria will be excluded). The cases need not be now alive nor current clinic cases.
2. The child must have been born in a Maryland hospital on or after January 1, 1950 and diagnosed with strabismus before January 1, 1963.
3. Single-born (confirmed from hospital records).
4. Selection will be by date of diagnosis beginning with most recently diagnosed cases.

The control group -- selected from birth certificates and matched with the cases in the following variables: (1) date of birth (next registered birth matching on all other relevant variables); (2) place of birth (same institution); (3) sex; (4) maternal age (matched within following categories: (a) under 20 years; (b) 20-34 years; (c) 35 years and over). All originally matched controls who die in the neonatal period will be replaced by appropriately matched neonatal survivors.

Information to be Collected:

(a) Cases and controls

In addition to the data necessary for matching from the birth certificate, detailed information about the study pregnancy, the study delivery, and the outcome of pregnancy--including infant weight--will be extracted from hospital records in a manner such that the abstracter will not know whether the individual involved was a case or control in order to insure against potential bias in recording of information. The following variables will be compared for the two groups: (1) birth weight distribution; (2) prenatal care; (3) prenatal complications; (4) past obstetrical history; (5) fetal presentation and position; (6) type of delivery; (7) length of labor; (8) toxemic, hemorrhagic, mechanical, and medical complications of delivery; (9) reported abnormalities of infant; (10) reported length of gestation.

(b) Cases only

Type of strabismus and muscle imbalance.

Analysis:

Comparison between cases and controls on variables under (a) above. Insofar as possible, different categories of strabismus and muscle imbalance will be studied separately.

Major Findings:

Data have been collected and put into IBM cards. The analysis has just begun.

Significance of Study:

To the knowledge of investigators this represents the first attempt to relate a wide range of prenatal and perinatal disorders to the occurrence of strabismus in children. In view of the fact that strabismus is often associated with the occurrence of amblyopia, the finding of a relationship between prenatal and perinatal disorders and strabismus might furnish clues how to prevent amblyopia.

Proposed Course of Project:

To finish collecting data, prepare tabulations, and analyze the data towards eventual publication of findings.

Part B included no.

Serial No. NDB (CF) - 64 B 1186
New Project
1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964--June 30, 1965

Part A

Project Title: Italian - American Tonometry Survey

Principal Investigator: John T. Schwartz

Other Investigator: Geoffrey G. Dell'Osso

Man-Years of Work

Total:	3/10
Professional:	1/5
Other:	1/10

Project Description:

This project was conducted jointly with the Epidemiology Branch, NINDB. The project description is indicated on the appropriate Individual Project Report of that Branch.

Part B included no.

Serial No. NDB (CF) - 63 B 1137
Continued Project since 1963
1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964--June 30, 1965

Part A

Project Title: A Study of Awareness, Attitudes and Performance in Reporting Persons to Blindness Registers

Principal Investigator: Hyman Goldstein

Other Investigators: Irving D. Goldberg;
Richard E. Onken, American Foundation for the Blind

Cooperating Units: Blindness register agencies of twenty-eight States with mandatory blindness registers

Man-Years of Work

Total:	1/10
Professional:	1/10
Other:	—

Project Description:

Objectives:

Investigate the awareness of, attitudes toward, and reporting performance regarding State registration of the blind on the part of ophthalmologists, eye-ear-nose-and-throat (EENT) specialists and optometrists. This will be accomplished (a) through interview of such reporting sources, (b) through interview of register agency staff, and (c) study of (1) written materials and other communications directed to ophthalmologists, EENT specialists and optometrists by State register agencies; (2) the forms and communications these agencies receive from ophthalmologists, EENT specialists and optometrists.

Study Populations:

1. State register agency directors and appropriate staff members including consulting or supervisory eye specialists in eight States.
2. Ophthalmologists, EENT specialists and optometrists in the 28 States selected.

Information to be Collected:

Information will be gathered from directors of register agencies and their staff members directly responsible for maintaining the register regarding:

1. The uses they make of the register.
2. The register law.
3. The operation of the register.
4. Services available to blind persons.
5. Written materials and other communications directed to ophthalmologists, EENT specialists and optometrists, particularly with respect to the type and extent of educational campaigns undertaken to inform potential reporting sources and professional associations of the desirability and need to report cases to the register.
6. The forms and communications received from such potential reporting sources.

Respondents will be randomly selected from directories of ophthalmologists, EENT specialists and optometrists in the selected States. These will be located in urban areas with population of 50,000 or more (as of 4/1/60) in order to reduce interviewing costs. It is recognized that limiting such interviews to reporting sources in such urban areas may also limit any generalization of findings to such areas only. Letters will be sent to each professional describing the purpose of the study and including documents of support from the respondent's professional society. Then a phone contact will be made to arrange an appointment at the respondent's convenience.

Interviews will take from one-half hour to an hour. The questionnaire will include closed and open-ended questions for probing attitudes toward registers and the reasons behind the attitudes. Due to differing roles regarding the register, there will be four separate questionnaires: one for directors of agency personnel and the staffs; a second for ophthalmologists who are advisors to the register agency; a third for other ophthalmologists and EENT specialists, and a fourth for optometrists. Interviewing will be conducted by trained interviewers under direction of a commercial research firm under subcontract. Interviewing is scheduled to start on or about May 1, 1965.

Analysis:

The basic hypothesis is that reporting of blind persons to register agencies by ophthalmologists and optometrists is based on their awareness of and a positive attitude toward registration of blind persons. Conversely, it is assumed that nonreporting is based on lack of information and/or on a negative attitude toward registration. In order to test this hypothesis, a typology of ophthalmologists, EENT specialists and optometrists will be constructed as follows:

1. Those who report all blind persons to the register agency.
2. Those who do not report all blind persons to the register agency since they are:

    (a) Not aware of the register and the need to report all cases of blindness.
    (b) Aware of the register but are unwilling to cooperate.

A comparison will be made between the selected States, based on the frequency and extent of their efforts to inform ophthalmologists, EENT specialists and optometrists of the need for the reporting of all cases of blindness.

A comparison will be made between the proportion of eye specialists and optometrists in each of these States who are aware of the register and information given by the register agency. The proportion who are aware of the register will then be related to the degree of effort in their State to inform them of the register.

Efforts will be made to determine: 1) what effect, if any, mandatory reporting had on completeness of reporting, and 2) the attitude of potential reporting sources toward mandatory reporting in States with or without such reporting laws.

It will also be determined whether ophthalmologists as compared to optometrists are more or less likely to report and why.

In all cases, an evaluation of the effort of State blindness agencies to orient potential reporting sources to the benefits of registration will be made so that results obtained may be viewed in relation to such orientation.

Since the basic hypothesis states that attitudes as well as awareness regarding the register influence the completeness of reporting, information collected on attitudes will be analyzed to determine those which lead to reporting and those which lead to nonreporting.

Since both closed and open-ended questions will be asked, codes and content analysis will be developed prior to data collection. The collected data will be tabulated by machine. The analysis of attitudes will include the construction of indices of attitudes toward the register agency, toward welfare agencies in general, toward blind persons, etc.

Major Findings:

Data have not been collected as yet.

Significance of Study:

It is clear that the proposed study will provide information of value in improving the completeness of reporting. It will reveal the extent to which attitudes hinder or encourage the achievement of complete reporting. The findings of this study will be evaluated by the Planning Group of the Model Reporting Area for Blindness Statistics. Therefore, after the research findings have been analyzed, the Planning Group will review these findings and, it is hoped, will be able to translate them into recommendations for implementation.

On the basis of the findings of this study the task of the Planning Group will be to make recommendations for the improvement of registers. The study report and its evaluation may also serve as a basis for the implementation of the findings through the consultative services offered by the staffs of the American Foundation for the Blind and the Biometrics Branch, NINDB.

Through the information gathered in this study a measure for evaluating the efforts of the register agency to encourage complete reporting may be provided by relating the communications directed to eye specialists and optometrists with the degree of awareness of these communications on the part of the eye specialists and optometrists. If it is found that these professionals are not aware of the communications, it is hoped that recommendations may be made to register agencies for improving communication practices based on the practices of more successful States and practices adopted by the Model Reporting Area for Blindness Statistics. If, on the other hand, it is found that reporting sources are aware of the register and the need to report all cases of blindness but do not do so, the reasons for nonreporting will be determined. If it is found that the reasons for nonreporting lie in objection to the procedures involved in the registration then it may be recommended that consideration be given by the register agency to changing such procedures, or that they inform reporting sources of the need for these procedures.

Proposed Course of Project:

To start to collect data, prepare tabulations and analyze the data with eventual publication of findings.

Part B included no.

Serial No. NDB (CF) - 62 B 968
Continued Project since 1962
1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964--June 30, 1965

Part A

Project Title: United States Mortality from Neurological and Sense Organ Diseases: A.P.H.A. Monograph Series

Principal Investigators: Irving D. Goldberg, Hyman Goldstein, Leonard T. Kurland, Gail R. Williams

Other Investigators: Jerome D. Schein, Gallaudet College
Rick Heber, University of Wisconsin

Man-Years of Work

Total:	3/20
Professional:	1/20
Other:	1/10

Project Description:

This is one of a series of monographs, sponsored by the American Public Health Association, which includes Members of the Biometrics Branch among the authors. This monograph will contain information on the mortality, morbidity, and epidemiology of neurological and sense organ disorders, including a review of pertinent literature already published. Detailed tabulations were prepared on mortality from various neurological disorders to be included in the monograph, and these will be incorporated, where pertinent, in the various chapters of the monograph. A meeting of the authors was held and plans for completion of the monograph were detailed. It is expected that the monograph will be completed in 1966.

Part B included no.

Serial No. NDB (CF) - 64 B 1187
New Project
1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964--June 30, 1965

Part A

Project Title: Study of Association between Factors of Pregnancy, Labor and Delivery and the Occurrence of Deafness in Children.

Principal Investigators: Hyman Goldstein and Irving D. Goldberg

Other Investigator: Geoffrey G. Dell'Osso

Cooperating Units: New York State Education Department
New York City Board of Education
New York State Department of Health
New York City Department of Health

Man-Years of Work

Total:	1/4
Professional:	1/5
Other:	1/20

Project Description:

Objectives:

To determine whether mothers of deaf children are characterized by an excess of selected prenatal and perinatal disorders as compared to mothers of the total population of live births surviving the neonatal period.

Study Population:

All children (about 900 cases) who meet the following four criteria:

1. Born during the period 1950-1959 and appointed prior to September 30, 1964 to a State school for the deaf by the Division for Handicapped Children, New York State Education Department.

2. Having deafness presumably prenatal or genetic in origin, or not otherwise known to be adventitious. For purposes of this study, a deaf person is defined as one who is totally deaf or has impaired hearing such that his auditory loss is 70 db. or greater in the better ear.
3. Single-born.
4. With birth certificates on file at the New York City Department of Health or the New York State Department of Health.

Control Group:

A stratified sample of 2 in every 1,000 recorded live births in New York during the years 1950-1959 inclusive.

Information Collected:

(a) Cases and controls: items appearing in birth certificates, viz. date and county of birth, sex of child, weight at birth, race of mother, age of mother, number of previous children, complications of pregnancy, complications of labor, operative procedure, Rh factor, presence or absence of congenital abnormality or birth injury.
(b) Cases only: diagnosis for each ear, date of earliest diagnosis, hearing loss in narrow speech range (500-2000 cps.) in db. for each ear.

Analysis:

Comparison between cases and controls on factors under (a). Separate analyses will be made for those cases of deafness who have other complications such as blindness, brain injury, etc. Insofar as possible, different categories of deafness as well as different degrees of hearing loss will be studied separately.

Major Findings:

Data still being collected.

Significance of Study:

This is, to the knowledge of investigators, the first attempt to relate a wide range of prenatal and perinatal disorders and the occurrence of deafness in children. If such association is found this may furnish information that would lead to improvement and prevention of certain types of deafness in children.

Serial No. NDB (CF) - 64 B 1187

Proposed Course of Project:

To finish collecting data, analyze and interpret them, and publish report.

Part B included no.

Serial No. NDB (CF) - 64 B 1188
New Project
1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964--June 30, 1965

Part A

Project Title: Study of Blindness Statistics in Egypt.

Principal Investigator: Hyman Goldstein

Other Investigator: Dr. Mohyi-Eldin Said,
University of Alexandria, Egypt, U.A.R.

Man-Years of Work

Total:	1/5
Professional:	1/5
Other:	—

Project Description:

Objectives:

To ascertain the feasibility of developing a blindness register on a pilot study basis in an Egyptian community as a mechanism for producing statistics in blindness (including severe vision impairment) needed for prevention and control activities. To determine to what extent modifications may be made in procedures developed by the Model Reporting Area for Blindness Statistics (MRA) in the United States that, when applied in Egypt, will still permit production of statistics comparable to those of MRA.

Study Population:

The study is being conducted in two urban areas (approximate population 125,000 persons) and in 22 rural villages (approximate population 125,000 persons). The study consists of three phases. In Phase I a random sample of approximately 1,000 households (approximately 5,000 persons) will be drawn from the urban study areas for house-to-house vision testing. The same size sample will be drawn from the rural study areas. With an estimated blindness prevalence rate of 15 per 1,000 population (using a definition of blindness comparable to that of the MRA), the survey should yield about 75 blind persons in the urban areas and the same number in the rural ones. In Phase III there will be a reexamination, about one and one-half

years later of persons in the original sample of 10,000 persons still resident in the study areas to determine new cases of blindness. In both phases, the examined population found not to be blind, is the control group. In Phase II, an attempt will be made to examine every resident in the study areas by persuading such residents to come to health centers voluntarily for a vision examination. This will establish a blindness register. Assuming again, an estimated prevalence rate of 15 per 1,000 population, approximately 3750 persons should be described as blind in this phase.

Control Group:

As mentioned above, in Phase I, the control group will be those found not to be blind by definition, or 9,850 of the 10,000 examined. The same will be true of Phase III. In Phase II which attempts to establish a blindness register, approximately 3750 persons will be cases and 246,250 persons will be controls.

Information Collected:

(a) Cases and controls: date and place of birth, sex, nationality, race, marital status, education, occupation, distance vision both uncorrected (or with present corrections) and with best correction for each eye, refractive, power of lenses, peripheral field vision where better eye with best correction has visual acuity greater than 6/60.

(b) Cases only: probable age of onset of severe vision impairment, history of severe ocular infections, operations, etc., with age at occurrence, blood relationship, if any, between parents, history of similar ocular conditions in blood relatives, near vision both uncorrected (or with present correction) and with best correction for each eye, intraocular pressure, cause of severe vision impairment (site and etiology), prognosis and recommendations for treatment, if any.

Analysis:

Comparison of total and cause-specific incidence and prevalence rates for the various age, sex, urban and rural, and other groups. Comparison of distributions of certain demographic variables in the cases and control groups.

Major Findings:

Data still to be collected.

Significance of Study:

This is considered to be the first attempt to apply standards of a highly sophisticated data collection procedures (MRA) to those of a developing country in order to determine what modifications may be made in such standards when they are applied in the developing country and still permit the production of comparable statistics. The utilization of such a "tool," in cooperation with scientific sampling methodology in under-developed countries has tremendous potential in the derivation of comparable international statistics of blindness. Since the prevalence rate of blindness in Egypt is one of the highest in the world, the development of comparable statistics for that country may provide valuable clues to the role of environment and other factors in the etiology of blindness. Specific information on age, sex and other demographic variables in the blind population will help pinpoint high risk groups who need to be screened for early signs of eye disorder.

Proposed Course of Project:

To collect, process, analyze and interpret data and to publish report.

Part B included no.

ANNUAL REPORT
JULY 1, 1964 THROUGH JUNE 30, 1965
EPIDEMIOLOGY BRANCH
NATIONAL INSTITUTE OF NEUROLOGICAL
DISEASES AND BLINDNESS

Introduction

Major changes in personnel have taken place within the Branch during the past year. On 1 November, Dr. Leonard T. Kurland, the Branch Chief, retired from the Commissioned Corps and left the Institute to take a civilian position. Dr. Jacob A. Brody, Chief, Epidemiology Section, Arctic Health Research Center, Anchorage, Alaska, will be transferred to Bethesda to become permanent Branch Chief on 1 July 1965. Dr. Brody is both a virologist and an epidemiologist.

Dr. J. Theodore Schwartz directed the Branch as Acting Chief during the last eight months of the Fiscal Year. Since he has primary responsibility for developing the program of collaborative and field research in ophthalmology, Dr. Schwartz was not charged with directing those Branch activities relating to the NINDB Research Center at Guam. Direction of the Guam program has been carried at a higher administrative level pending the arrival of Dr. Brody.

There were several other changes in our professional staff during the year. Drs. Dwayne M. Reed, Bernard M. Abrams and Yoshiro Yase left the Branch, and Drs. Richard L. Need, Asao Hirano, Antonio Stazio and Gail R. Williams will have left by the end of July 1965. Mr. Chris Plato and Dr. Teresita Elizan were transferred from Guam to Bethesda in August 1964 and April 1965 respectively. Dr. Kwang Ming Chen, a neurologist from Taiwan, joined the Branch at Guam in August 1964. Most of the vacancies created by the turnover of personnel have been left unfilled in order to permit recruitment of staff with training in those disciplines required for Dr. Brody's program.

Rapid, continual turnover in personnel has been a recurrent problem for the Branch and has been discussed in several annual reports. The shaping of a new personnel structure and the establishment of an effective research team will be objectives of major importance during the next fiscal year.

The chief areas of activity of the Branch during the period of this report have been: 1. Further development of the program of collaborative and field research in ophthalmology, 2. Further studies in neuropathology, including fine structure of cerebral fluid accumulation, 3. Continued investigation of neurologic and other medical problems in Guam, 4. Liaison and coordination

of further studies on the toxicity of cycads, and 5. Multiple sclerosis survey in New Orleans. These activities will be discussed in the above order. Comments on program plans and proposed future objectives are included in the narrative sections where appropriate.

1. Collaborative and Field Research in Ophthalmology

1-A. CHRONIC SIMPLE GLAUCOMA. A major portion of our investigative field work has been directed toward understanding the importance of some of the variables affecting the reliability of tonometric screening procedures.

Our analysis of the comparability of a sample of certified Schiotz tonometers was completed [NDB (CF) - 63 E 1123]. This field comparison permitted the identification of a small number of Schiotz tonometers which functioned at the middle of the "performance distribution." As one result of this study we were able to select for further field use a small number of instruments known to have been in approximate agreement.

A pilot study was performed at Soldier's Home, Washington, D. C. during which the feasibility of attempting Goldmann applanation measurements as a field procedure was demonstrated. The Goldmann applanation tonometer is considered to provide a more accurate measurement of intraocular pressure than the Schiotz indentation tonometer, and can be adapted to a sitting examination with a slit lamp or to a supine examination using an operating microscope. Following the initial feasibility study, highly successful surveys were carried out on two different "total" adult populations: Italian-Americans residing in Nesquehoning Pennsylvania and American Indians at the Colorado River Indian Reservation, Parker, Arizona. In both surveys tonometry was performed on each examinee with the Schiotz tonometer, and with the Goldmann applanation tonometer in both the sitting and supine position. These studies will provide a comparison of the screening results obtained with Goldmann and Schiotz tonometers, and will also define the influence of postural differences on the Goldmann applanation screening technique in the two study communities.

In the Parker, Arizona study we collaborated with a team of investigators from Washington University, who performed a battery of clinical examinations for glaucoma. One objective of this collaboration was to appraise the relative validity of our three tonometric screening procedures.

By selecting Italian-American and American Indian populations for our tonometry studies, we have also acquired pilot descriptive data pertaining to our additional interest

in investigating the nature of the association between chronic simple glaucoma and chronic vascular disease. [See project reports No. NDB (CF) - 63 E 1122, 64 E 1255, and 64 E 1256].

The extent to which variations in tonometry technique can influence the outcome of a population survey was also estimated by a graphic method in a recent paper by Dr. Schwartz. It was shown that a systematic error of only 2 millimeters of mercury between two studies or two techniques can in itself alter the proportion of positive screenees by as much as 60 percent. A single, specific and carefully defined tonometry technique must find broad acceptance in order to acquire comparable geographic data on the frequency of ocular hypertension.

Wide acceptance of carefully defined field techniques must precede worthwhile field investigations; accordingly, attempts to arrive at acceptable standard procedures must receive early attention. It is proposed that the Institute enthusiastically support efforts to seek agreement on field techniques among those who are interested in epidemiologic field studies of glaucoma. Dr. Schwartz is scheduled in June to discuss "Problems relating to the epidemiology of chronic simple glaucoma" at a symposium on glaucoma screening in London. This symposium has been organized by Professor E. S. Perkins of the University of London and will be attended by European epidemiologists and ophthalmologists. This forum may provide an opportunity to explore possible ways to seek agreement on field standards.

1-B. GENETIC STUDIES. An investigation of the relative role of genetic or environmental influence underlying the development of chronic simple glaucoma has begum. During the past year, a registry containing over 200 pairs of local twins has been developed by the ophthalmology activity. When clinical privileges and personnel become available, the staff will undertake a comparison of concordance among monozygous and dizygous twinships with regard to several clinical parameters of glaucoma. A comparison of the response to steroid provocation will be included in the glaucoma evaluation.

We hope to expand the twin registry and develop it as a source of genetic information on other ocular disorders. Now under consideration are genetic investigations of refractive error employing ultrasonic measurements of ocular dimensions, investigations of motility imbalance and investigations of certain physiologic responses. Our twin population may also be made available to investigators in other disciplines.

1-C. MALIGNANT NEOPLASMS OF THE EYE. A study of U. S. mortality experience with malignant neoplasms of the eye (Rubric 192)

has been initiated in collaboration with the Biometrics Branch, NINDB. During 1959-61, there were filed in the United States 1,108 death certificates in which the primary cause of death was assigned to Rubric 192. The data from these records will be analyzed for certain epidemiologic features such as age, race, sex and geographic distribution. An attempt will be made to characterize these features by the histologic type of tumor. It is anticipated that additional information will be solicited on certain cases, and where necessary attempts will be made to recover surgical or autopsy specimens. Recovered pathologic material would be examined at the Armed Forces Institute of Pathology.

1-D. TELEVISION OPHTHALMOSCOPY. The television ophthalmoscope is of interest to the Branch because of its potential value in epidemiologic and collaborative research and also because of the probable need for collaborative effort in its further development. The Institute held a Workshop in February 1965 to explore the desirability of initiating and supporting a program of further development of the television ophthalmoscope. The Workshop was organized and moderated by Dr. Schwartz. Participants represented many disciplines: engineering, electronics, biophysics, ophthalmology, neurology, epidemiology, experimental medicine and administration. After discussing several applications of the instrument as well as clinical requirements, problems of system design and possible methods of approach, the consultants voiced a strong recommendation that the Institute proceed to coordinate and participate in further development of television ophthalmoscopy. The Workshop participants emphasized that a collaborative approach to the overall system design would be necessary.

Beyond achieving its immediate objectives, this Workshop also provided an important point of view which has far reaching implications relative to our program planning in ophthalmic collaborative and field research. The television ophthalmoscope is only one example of an ophthalmic application of the products of recent technologic advancement. The refinement of such applications from the stage of instrument development to clinical usage requires the collaboration of science, industry and medicine as well as the collaboration of multiple disciplines within. The interdisciplinary requirements for development of such instruments may now be exceeding the capacity of single medical or industrial organizations. Accordingly, our activity faces a new responsibility in collaborative ophthalmic research. It is strongly recommended that most serious consideration be given to the organization of a Section on Instrument Development within our ophthalmic collaborative and field activity.

1-E. COMMUNICABLE DISEASES OF THE EYE. The initial selection of objectives for epidemiologic investigation by the Branch was guided by our interest in diseases which are of major importance to our total population [NDB (CF) - 63 E 1122]. Chronic ophthalmic disorders occupy the position of highest priority in this regard, and our earliest efforts have been so directed.

During the past two years we have had many requests for consultation on problems relating to external eye disease, including outbreaks which were potentially of viral origin [NDB (CF) - 63 E 1119]. Although basically our research is being directed toward our responsibility in the area of chronic disease, we have become keenly aware of additional obligations with regard to outbreak epidemiology and to external diseases of the eye. To provide a comprehensive program in epidemiology, it is important that we expand our capability in these areas.

1-F. PROGRAM PLANS AND PROPOSED FUTURE OBJECTIVES OF THE COLLABORATIVE AND FIELD PROGRAM IN OPHTHALMOLOGY. The Ophthalmology Projects activity has been charged with the responsibility of developing a balanced program of collaborative and field research in ophthalmology.*

The obligations of this activity with regard to direct operation, consultation and coordination are numerous and will continue to grow in importance. Expanding obligations in the field of communicable eye disease, in the development of ophthalmic instrumentation, and in other areas have been discussed in the preceeding narrative. In the near future we anticipate still greater participation and consultation in instrumentation field trials, trachoma control and collaborative population studies.

A viable program of collaborative and field research in ophthalmology, operating centrally within the National Institute of Neurological Diseases and Blindness, should contribute to the progress of ophthalmic research. The execution of our responsibility requires that the Ophthalmology Projects activity be provided with corresponding authority and support. Since the program in Ophthalmology was instituted within the Epidemiology Branch approximately two years ago, its professional staff has consisted of a single billet: Head, Ophthalmology Projects. Until 1965 this activity functioned without a committed budget.

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*Billet description, Head, Ophthalmology Projects, Epidemiology Branch, NINDB. PHS-4392, August 12, 1963.

The Division of Collaborative and Field Research, NINDB, is currently undergoing personnel changes throughout its structure, and its organization is in a phase of transition. It is respectfully suggested that this is the appropriate time for evaluation of the Institute's objectives in Ophthalmic Collaborative and Field Research and for careful consideration of the organizational structure, authority, and support required to accomplish these objectives.

2. The fine structure of cerebral fluid accumulation

Previous light and electron microscopic studies by Dr. Hirano revealed that implants of cryptococcal polysaccharide in rat brain produced an accumulation of fluid which was distributed selectively in white matter, between the myelinated nerve fibers, nonmyelinated nerve fibers and glial cell processes.

Direct extension of the material into cytoplasm through ruptured glial and neuronal plasma membranes has now been observed, exclusively in tissue surrounding the original implant. A few macrophages appeared at the implant site, forming multiloculated intracytoplasmic inclusions which were denser than the extracellular material. These studies are elucidating some of the dynamic processes involved in transfer of fluid from extracellular compartments in the brain.

A unique reaction of oligodendroglia to certain polysaccharides which may have some relationship to the classical alterations known as acute swelling and mucoid degeneration of oligodendroglia has also been observed by Dr. Hirano and co-workers. About one week after pellets of cryptococcal capsular polysaccharides mixed with graphite were implanted in the rat forebrain, the interfascicular oligodendroglia became distended by numerous vacuoles filled with polysaccharide-rich fluid. These ballooned cells persisted at least three months.

Direct "invasion" of polysaccharide-rich fluid into astrocytes through ruptured plasma membranes was also seen at the site of implant, with persistence of intracellular polysaccharide for as long as 100 days. Considerable amounts of fluid were engulfed by subependymal phagocytes and smaller amounts entered the cytoplasm of ependymal cells. Phagocytes with engulfed fluid were able to traverse the ependymal barrier into the lumen of the ventricle.

These techniques have also been applied in studies of experimental leukoencephalopathy and of cerebral cryptococcosis. Cryptococcal capsule, cell wall and purified capsular polysaccharide were extremely resistant to digestion, even when engulfed by phagocytes in the brain, and caused little inflammatory reaction. Cryptococcal organisms,

which often cause vigorous inflammation in other organs, are known to show a predilection for the brain where their infection is chronic and progressive in character.

3. Continued investigation of neurologic and other medical problems in Guam

In April 1964 the Guam genetic registry survey on ALS-PD was completed. Analyses of the genetic, epidemiologic, and neurologic data from 1957 to 1964 have been completed and are presently being prepared for publication. (See separate detailed report by Dr. Elizan to Dr. Caveness.) Dr. Hirano is preparing a supplementary report on the neuropathology of the cases reviewed by Dr. Elizan.

A summary of the above data will be presented by Drs. Elizan and Hirano at the Symposium on Motor Neuron Disease, International Congress of Neuropathology, in Zurich, Switzerland, September 1965.

Continued evaluation of neurological problems in Guam, including diseases other than ALS-PD, has been done by Drs. Elizan, Abrams, Yase and Chen. Twenty-seven cases of myotonic dystrophy occurring in four families, will be reviewed and prepared for publication.

Case-finding studies on non-Chamorros in Guam, and in other Mariana Islands, and in the Caroline Islands are being continued. Sections of Yap, Palau, Panope, and Truk have been visited; a possible case of PD with suggestive motor neuron features was found in Panope. Previous "cases" and "suspects" in the other islands have been followed. The confirmation of the Guam ALS-PD among non-Chamorros awaits histological confirmation at this time.

The training of native medical practitioners in the various Caroline Islands in neurological screening techniques by the NINDB staff on Guam was started in March 1965, and is thought to be essential to any projected case-finding surveys of the islands.

Diabetes in Guam was studied by Dr. Need. This data is being prepared for publication.

Genetic and serological investigations on Chamorros and Carolinians have been completed by Mr. Plato.

Uric acid determinations on Chamorros and Carolinians were initiated by Dr. Need and will be completed by June 1965.

4. Liaison and coordination of further studies on the toxicity of cycads

Liaison is maintained by Dr. Whiting with more than thirty collaborators on cycad research working in twelve research centers throughout the world. The Branch has continued to assume responsibility for procurement of supplies of raw plant material and extracts for distribution to researchers. Raw material from Cycas circinalis has been obtained mainly from the Department of Agriculture in Guam. With the assistance of the South Puerto Rico Sugar Company Trading Corporation, supplies of tubers and leaves of Zamia debilis are now being obtained from the Dominican Republic.

Advances in animal research include the production with either cycad meal or cycasin of malignant tumors at multiple sites in rats and guinea pigs, liver damage in germ-free rats with the oral feeding of the aglycone from cycasin, malignant tumors at multiple sites in rats with the feeding of root from the cycad Zamia spp. obtained from Florida, and the demonstration in rats that milk from a lactating animal fed cycad meal produced liver damage in the suckling. Dr. Magee, Toxicology Research Unit, Surrey, England, produced kidney neoplasms by a single intraperitoneal injection of cycasin in day-old rats.

Drs. Matsumoto, Nagahama, and Larson at the University of Hawaii synthesized the acetate of methylazoxymethanol (MAM), the toxic portion of the various glycosides isolated from different genera of cycads. With commercial production of MAM-acetate and radioactively labelled derivatives, experiments which were previously impossible can now be implemented [NDB (CF) - 63 E 1112].

Work by Teas, Saxe, and Saxe at the University of Georgia demonstrated that cycasin induced chromosome breaks in Allium cepa. (Science, in press).

A new geographic area for research, the Dominican Republic, was visited by Drs. Whiting, Tsai (NHI), Mason and Frederickson (Mason Research Institute) for investigation of a lameness in cattle reportedly due to ingestion of the cycad, Zamia debilis. A film was made showing several afflicted animals, and biological specimens were obtained from six animals by Drs. Mason and Frederickson. Histological study of the material from these cattle continues with the aid and consultation of Dr. Innes (Bionetics Lab., Inc.).

Dr. Clifford Gallagher, Veterinary Pathologist at the University of Sydney, Australia, has agreed to examine histologically material from cattle afflicted with "cycad paralysis" in Papua, New Guinea and Queensland, Australia. Dr. Hall of Queensland has a publication in preparation reporting demyelination of both

the fasciculus gracilis and the dorsal spinocerebellar tracts of a cow afflicted during feeding trials with leaves of the cycad Macrozamia lucida [NDB (CF) - 63 E 1111].

The report of the Third Conference on the Toxicity of Cycads (April 1964) was published in Federation Proceedings 23:1337-1388 (1964). A Fourth Conference was held at the National Institutes of Health on 15 April 1965, sponsored by the National Institute of Neurological Diseases and Blindness, the National Institute of Arthritis and Metabolic Diseases and the National Cancer Institute. The National Cancer Institute provided funds for honorariums, stenotypist and publication.

In his final contract report, Dr. Forgacs, Suffern, New York, especially urged additional research on four fungi isolated from cycad plant material: Aspergillus niger, Aspergillus flavus Link, Penicillium purpurogenom, and Microsphaerella spp. The latter predominates on and within the leaves and tubers of Zamia from both Florida and the Dominican Republic.

5. Multiple sclerosis survey in New Orleans

A follow-up of previous population studies of multiple sclerosis conducted in New Orleans in 1949 and 1951 is underway by Dr. Stazio. The objectives of the study are (1) to evaluate the accuracy of the 1949 and 1951 case selections; (2) to provide information on survivorship patterns and the natural history of multiple sclerosis; (3) to clarify certain aspects of the distribution of the disease in New Orleans; and (4) to determine prevalence and incidence trends over the past 20 years.

Serial No. NDB (CF) - 54 E 102
1. Collaborative & Field Research
2. Epidemiology Branch
3. Agana, Guam

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Population survey and case-finding study of ALS-PD complex in the Mariana Islands other than Guam and the Caroline Islands

Principal Investigators: T. S. Elizan
Y. Yase
B. M. Abrams
C. Van Nuis
R. L. Need

Other Investigators: D. M. Reed
J. M. Torres
F. G. L. Guerrero
L. T. Kurland

Man Years
Total: 1/4
Professional: 3/16
Other: 1/16

Project Description:

Saipan, Tinian, Rota, and the Northern Marianas group of islands have been totally surveyed for the presence of ALS-PD complex. The high prevalence of this disease entity was previously considered to be confined to the Chamorro people of Guam, and the leading hypothesis as to etiology involved an hereditary factor operating in the Chamorro population. A nonChamorro (Carolinian) population sharing a common environment with a vulnerable or afflicted Chamorro population exists in Saipan and in the Northern Marianas. Saipan has a total population of approximately 7,200 people, 1,100 of whom are Carolinians, and the rest, Chamorros. These two ethnic groups reside in adjacent, and often in the same, communities. The Northern Marianas have five islands inhabited by both Carolinians and Chamorros, the former outnumbering the latter.

There is evidence at this time that Carolinians in Saipan may have a high prevalence of ALS (100/100,000) - which may be as high as the prevalence rate among Chamorros in Guam (127/100,000, as of June, 1963).

There is also evidence that Chamorros in the rest of the Marianas, other than Guam, (Saipan, Rota, Northern Marianas) have likewise a high prevalence (50/100,000), though not as high as that of the Chamorros in Guam. These findings reflect a probable significant role of environmental factors on the development of the disease in genetically vulnerable racial groups. Continuing clinical studies, including hospitalization and diagnostic work-up of suspicious cases, and postmortem follow-up of definite cases, are being made. Blood studies of these populations, including blood grouping, haptoglobins, hemoglobins, and carbonic anhydrase determinations, are being done by Mr. Chris Plato, geneticist, and constitute several separate projects. More detailed epidemiological study of these islands will be undertaken in the future.

Part B included: No

Serial No. NDB (CF) - 54 E 103
1. Collaborative & Field Research
2. Epidemiology Branch
3. Agana, Guam & Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Clinical studies on ALS-PD complex of Guam

I. General medical aspects

Principal Investigators: R. L. Need
T. S. Elizan

Other Investigators: Y. Yase
L. T. Kurland

Man Years

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Heretofore, the clinical evaluation of ALS-PD patients in Guam had been solely concentrated on the nervous system. This study was started to evaluate (a) the general medical condition of these patients, and (b) the occurrence of other associated diseases and/or abnormalities. All living ALS-PD patients and matched controls in the registry panels are given a thorough general physical examination, and the following laboratory work-up:

1. total serum proteins, A/G ratio, bilirubin, cephalin flocculation test, to evaluate liver function;

2. cholesterol and serum lipids are being done because of the apparent low incidence of arteriosclerotic changes in the heart and in the brain;

3. uric acid, Bentonite flocculation test for rheumatoid factor and glucose levels are being determined in a joint project with the staff of NIAMD;

4. blood urea nitrogen, for gross evaluation of renal function;

5. sodium and chloride;

6. serology; and

7. EKG, to determine any abnormalities. Russian workers have reported nonspecific EKG changes in ALS.

The general pathology of organs outside of the central nervous system has not been adequately studied in the Guam ALS-PD cases. There are 32 autopsied cases of ALS-PD (1961-1963) whose nonnervous tissues and organs are available for gross and microscopic pathological examination. Special attention will be focused on the heart, liver, lungs, and kidneys.

An evaluation of the total pathology of ALS-PD, correlated with the general medical picture, will be attempted.

Material from this project will be incorporated in the paper by Dr. Elizan noted in Part II.

II. Neurological aspects

Principal Investigators: T. S. Elizan
B. M. Abrams
C. Van Nuis

Other Investigators: Y. Yase
K. M. Chen
R. L. Need
L. T. Kurland

Man Years

Total:	1/4
Professional:	3/16
Other:	1/16

Project Description:

Detailed clinical analysis of motor neuron disease and Parkinsonian syndrome with associated organic mental changes, as found in Guam, will be done on the case material of the NINDB from 1957 to 1964. A total of 104 ALS cases and 72 PD cases are available for study.

Evaluation of the following items will be attempted:

1. onset and sequence of development of symptoms and signs; patterns of involvement of the CNS;

2. interrelationships between the PD picture and motor neuron features; peculiarities of the "mixed" types of cases in contrast with the "pure" types;

3. differences and similarities between the "Guam disease" and the classical motor neuron disease and Parkinsonian syndrome as observed in the West;

4. detailed characteristics of the "dementia" in the PD cases;

5. clinico-pathological correlations whenever possible;

6. analysis of age and sex characteristics and duration of illness with clinical progression of the disease; and

7. associated metabolic and other medical diseases with the PD and/or ALS picture.

An additional group of living patients consisting of (a) those with "pure" Parkinsonian features resembling, in some respects, that seen classically in the West, with no dementia, and (b) those who exhibit, at this time, only suspicious or indefinite Parkinsonian or motor neuron features, will be further studied and followed up, to clarify a suspected spectrum of this disease complex, with all its "intermediate" forms, and to further analyze the actual evolution of the disease process as it becomes more definitive.

An initial draft of this paper has been completed.

Part B included: No

Serial No. NDB (CF) - 55 E 201
1. Collaborative & Field Research
2. Epidemiology Branch
3. Agana, Guam

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Epidemiological Aspects of ALS/PD Complex on Guam

Principal Investigator: D. M. Reed

Other Investigators: C. C. Plato
T. S. Elizan
B. M. Abrams
R. L. Need
J. M. Torres
M. T. Cruz
F. C. L. Guerrero
L. T. Kurland

Man Years

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

A. ALS-PD Case Control

Every ALS or PD patient included in the registry from 1958 to 1963 was matched with a "normal" control for age, sex, and village of residence. Patients and controls who are still alive at this time were studied for differences in diet, microenvironment, socio-economic status, occupation, and disease history. No significant differences were found between cases and controls.

B. Other Epidemiological Studies of ALS-PD

Using the ALS-PD cases ascertained during the past five years, village of residence, prevalence and incidence rates were calculated. These data are strikingly similar to the data collected by Kurland et al. in 1953. The incidence rate for ALS was found to be 53 per 100,000 population. The rate for PD was approximately 19 per 100,000.

Tabulation of rates by village of birth using the 1930 population census, and detailed checking of the villages of birth of all cases was done. There was an indication of a north to south increase in incidence of ALS but not PD.

Death certificates were checked for the past 10 years for deaths due to ALS, PD, or suspiciously related neurological diseases. The known ALS cases who were alive in 1953 are also being traced. ALS accounted for 10% of all adult deaths, PD for 5%, as compared with 1 out of 1000 in the U.S.

Part B included: No

Serial No. NDB (CF) - 59 E 604
1. Collaborative & Field Research
2. Epidemiology Branch
3. Agana, Guam

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Electroencephalographic studies on ALS-PD cases on Guam.

Principal Investigators: T. S. Elizan
B. M. Abrams
K. M. Chen

Other Investigators: K. V. Mathai
J. M. Torres
F. G. L. Guerrero
L. T. Kurland

Man Years
Total: 1/4
Professional: 1/8
Other: 1/8

Project Description:

The frequency and degree of electrical abnormalities in the Guam ALS and PD cases (224 EEG's on 119 patients and 34 controls) have been analyzed. They will be incorporated into the before mentioned paper by Dr. Elizan (see Serial No. NDB (CF) - 54 E 103, part II).

Part B included: No

Serial No. NDB (CF) - 60 E 705
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland & Agana, Guam

PHS - NIH
Individual Projeet Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Population survey and case-finding study of ALS-PD complex in the Caroline Islands.

Principal Investigators: T. S. Elizan
B. M. Abrams
Y. Yase
C. Van Nuis
R. L. Need

Other Investigators: D. M. Reed
J. M. Torres
F. C. L. Guerrero
L. T. Kurland

Man Years
Total: 1/4
Professional: 3/16
Other: 1/16

Project Description:

A 1962 survey of Saipan done by Mathai et al. showed a possibly higher prevalence of ALS among the Carolinians than among the Chamorros - a finding that contradicted a previous impression that the ALS-PD complex was prevalent only among the Chamorros of Guam. Study of the migration waves of the Saipanese Carolinians indicated that the majority came originally from the western group of islands in Truk, and the eastern group of islands in Yap, although some originated from the Truk Atoll or other islands. Additional information has been discovered in the files of the USS Whidbey suggesting that Palau district may have a high frequency of neurologic disorders. Thus, it became necessary to study the incidence of ALS-PD in the population of the Caroline Islands. The role of external environmental factors which might not be confined to the Marianas, in the etiology and development of ALS-PD complex, needed to be further explored and studied.

Preliminary case finding surveys among a portion of the adult population was carried out in the entire Truk Atoll, the

Western Islands, Namonuito and Hall Islands of the Truk District, the Mortlocks, and Yap. Only one case of possible ALS confined to the spinal cord has been noted. However, without a total population survey of the entire Caroline Islands, it is not possible to draw definitive conclusions regarding the incidence of any neurological disease. The main problem regarding a total population survey is transportation to these small and out-of-the-way islands. In the meanwhile, members of the staff have continued previous visits to some of the larger island districts to judge the feasibility of surveys on a "live in" basis, and to do screening neurological examinations on limited portions of the native populations. Sections of Yap, Palau and Panope have been visited. A possible PD case with suggestive ALS component was noted in a Carolinian woman from Panope.

The training of local medical practitioners from the various islands in neurological screening techniques by the NINDB staff in Guam for one to two week periods, is thought to be an essential part of any projected intensive surveys of the islands; this plan has been started in March, 1965.

Part B included: No

Serial No. NDB (CF) - 60 E 715
1. Collaborative & Field Research
2. Epidemiology Branch
3. Agana, Guam

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Survey of Diabetes mellitus and gout in Guam.

Principal Investigator: R. L. Need

Other Investigators: T. A. Burch
NIAMD
W. O'Brien
Yale University Medical School
T. S. Elizan
D. M. Reed
C. C. Plato
L. T. Kurland

Cooperating Units: NIAMD
Yale University Medical School , New Haven, Conn.
Guam Department of Public Health , Agana, Guam

Man Years
Total: 1/2
Professional: 3/8
Other: 1/8

Project Description:

In 1962 a preliminary survey of two villages in Guam suggested a high incidence of hyperuricemia and hyperglycemia.

A survey of the population aged 21 and older was made in three villages in 1963, with the following results:

1. an incidence of diabetes mellitus of 33% in patients with PD (7 out of 21 cases), a higher rate than one would expect in this age group;

2. patients with ALS did not have a high incidence of diabetes;

3. although diabetes may be slightly more common in the Chamorro population than in American populations, it

is a mild adult-onset type of diabetes commonly associated with obesity and the female sex; and

4. no correlation was found between hyperuricemia and neurologic diseases in Guam.

Part B included: No

Serial No. NDB (CF) - 61 E 832
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Studies on the natural history of multiple sclerosis.

Principal Investigator: L. T. Kurland

Other Investigators: G. W. Beebe
National Research Council
J. F. Kurtzke
Veterans Administration Hospital
B. N. Nagler
Lynchburg Training School and Hospital

Cooperating Units: National Research Council, National Academy of Sciences, Washington, D. C.
Veterans Administration Hospital, Washington, D. C.
Lynchburg Training School and Hospital, Lynchburg, Virginia

Man Years:

Total:	1/8
Professional:	1/16
Other:	1/16

Project Description:

The study has three distinct phases: (1) progression from acute, idiopathic optic neuropathy to multiple sclerosis; (2) clinical patterns in the course of multiple sclerosis and the prognostic significance of a wide variety of factors, present at onset, and of subsequent residence; and (3) epidemiology, with attention to prior medical history, sociologic variables, and many factors in the natural environment, especially climatologic and other correlates of geography.

Phase (1) has been completed and the report was published. The other phases are underway based on comparisons of approximately 400 patients and an equal number of matched "controls."

Another study was made of 183 patients with an acceptable diagnosis of unilateral papillitis and unilateral or bilateral retrobulbar neuritis. These patients were followed-up through a variety of sources: 21 were judged "definite multiple sclerosis," 3 "possible multiple sclerosis," and the remaining "not multiple sclerosis." Thus, 13.1 percent (24/183) of the patients developed multiple sclerosis during the follow-up period, an incidence considerably lower than that reported by other investigators.

Part B included: Yes

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Nagler, B. N.; Kurland, L. T.; Auth, T. L.; Beebe, G. W.; Kurtzke, J. F.; Lessell, S. and Nefzger, M. D.: Studies on the natural history of multiple sclerosis. I. Objectives, designs, and methods. Acta Neurologica Scandinavica, Supplement (in press).

Kurland, L. T.; Auth, T. L.; Beebe, G. W.; Kurtzke, J. F.; Lessell, S.; Nagler, B. N. and Nefzger, M. D.: Studies on the natural history of multiple sclerosis. II. Progression from optic neuropathy to multiple sclerosis. Acta Neurologica Scandinavica, Supplement (in press).

Honors and Awards relating to this project: None

Serial No. NDB (CF) - 62 E 953
1. Collaborative & Field Research
2. Epidemiology Branch
3. Agana, Guam & Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: A serological and hematological genetic survey of Chamorros in Guam.

Principal Investigator: C. C. Plato

Other Investigator: M. T. Cruz

Man Years

Total:	1/2
Professional:	1/8
Other:	3/8

Project Description:

The object of this study is to obtain data on the distribution of the ABO, MNS, Rh, Kell, Duffy, Diego and other blood groups among the Guamanians, and to compare this distribution with the distribution of neurologic diseases in this population.

Over three hundred specimens have been collected from five Guamanian villages. In addition to the blood grouping studies, a portion of the red cells were hemolyzed for hemoglobin investigations. Serum protein electrophoresis was also performed for the determination of the frequencies of the various haptoglobin types among the Chamorros.

Concurrently with the population survey the same serological and hematological tests were performed on a number of ALS/PD patients. The results of the patients and random population samples will then be compared in order to determine whether there are associations between ALS/PD and any of the blood groups or haptoglobin types.

This project was formerly titled "Blood group genetic survey of Chamorros in Guam."

Part B included: No

Serial No. NDB (CF) - 62 E 957
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Pathological features of Amyotrophic Lateral Sclerosis and Parkinsonism-Dementia Complex, both endemic diseases on the island of Guam.

Principal Investigator: A. Hirano

Other Investigators: N. Malamud
Langley-Porter Neuropsychiatric Institute
L. T. Kurland
J. M. Torres

Cooperating Unit: Langley-Porter Neuropsychiatric Institute
San Francisco, California

Man Years
Total: 1/2
Professional: 3/8
Other: 1/8

Project Description:

A continuation of the clinico-pathological investigations which have been under way in an effort to provide further evidence that may aid in the evaluation of these disorders.

The results of this study will be presented at the Vth International Congress of Neuropathology, Zurich. Theme 11a, Amyotrophic Lateral Sclerosis, Parkinsonism-Dementia Complex, and Kuru Disease in the Western Pacific Islands. In the afternoon session, Monday, 30 August 1965.

Part B included: No

Serial No. NDB (CF) - 62 E 959
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Further studies on the CA_1C variant of carbonic anhydrase enzyme.

Principal Investigator: R. Tashian
University of Michigan

Other Investigator: C. C. Plato

Cooperating Unit: University of Michigan , Ann Arbor, Michigan

Man Years
Total: 1/32
Professional: 1/32
Other: 0

Project Description:

In our biochemical and enzymatic studies we were fortunate to discover a new variant (CA_1C) of carbonic anhydrase. This we showed to be inherited and to be controlled by a single autosomal gene present in the Chamorros of Guam and Saipan, but apparently not in the Carolinians.

The purpose of this study is twofold: (1) to test a random number of Chamorros in order to determine the frequency of the CA_1C gene in this population; and (2) to obtain larger quantities of blood from carriers of this gene in our effort to isolate this substance and study its enzymatic and biochemical properties in greater detail.

Over three hundred Chamorro blood specimens have been collected and are being tested for the presence of the CA_1C variant. Also, biochemical studies are being carried out for chemical identification and isolation of this enzyme.

Part B included: No

Serial No. NDB (CF) - 62 E 961
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Five year registry study of ALS-PD cases of Guam.

Principal Investigator: C. C. Plato

Other Investigators: T. S. Elizan
D. M. Reed

Man Years

Total:	7/8
Professional:	3/4
Other:	1/8

Project Description:

The role of heredity in the etiology of ALS has been a debatable issue since the discovery of the disease. Guam, with its peculiarly high prevalence of ALS presents an excellent testing ground for the genetic hypothesis. In 1958, a point survey of patients and controls was conducted by Dr. Robert Krooth, a geneticist and former member of this Branch. Even though the results of that survey strongly suggested that ALS occurred more often among the sibs of patients than one would expect by chance alone, the data failed to show the same trend among the offspring and parents of patients. A similar analysis of the PD cases failed to offer conclusive indications of familiality mainly because of the limited amount of data available at that time. In view of the inadequacy of the point survey, a new prospective study was initiated in 1958 by Dr. Krooth, with the following objectives:

1. to determine whether a familial concentration of the disease exists in both ALS and PD;

2. to determine if there is a significant tendency for a parent-offspring aggregation in these diseases; and

3. to conduct general comparisons among the relatives of the patients and controls.

The basic principle of the prospective study was the accumulative registering of each and every new patient and a suitable control during a 5-year period, and if necessary, during a 10-year period, after the initiation of the study. The living parents, sibs, offspring, and spouses of both patients and controls were likewise registered. Complete family histories were also recorded.

Case finding among the relatives of patients and controls consists of a thorough neurological examination; any suspicious or doubtful "cases" are reexamined by the staff neurologists, and either confirmed or rejected as a case.

Because of the growing clinical, pathological and statistical indications that ALS and PD are two forms of the same disease spectrum the data for ALS and PD were pooled and analyzed as if we were dealing with one disease, ALS/PD.

After five years of existence the patient-control panels were closed and over 80% of 2,000 relatives registered were located and given complete neurological examinations.

The collection of the data has been completed and all the necessary calculations were concluded. The results are presently being evaluated. A final report on this project will be prepared shortly.

Part B included: No

Serial No. NDB (CF) - 62 E 962
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Glucose-6-Phosphate Dehydrogenase Deficiency and other Sex-Linked Traits

Principal Investigators: C. C. Plato
M. T. Cruz

Other Investigator: L. T. Kurland

Man Years

Total:	1/32
Professional:	0
Other	1/32

Project Description:

The original project of the investigation of the glucose-6-phosphate dehydrogenase deficiency on Guam has been expanded, with the inclusion of the study of two other sex-linked traits: Xg^a blood group and red-green color blindness.

Two hundred and forty-six male students from both high schools of Guam have been tested for glucose-6-phosphate dehydrogenase deficiency and color-blindness. Of these, 109 were also typed for the new blood group Xg^a. The gene frequency of glucose-6-phosphate dehydrogenase deficiency is indeed very low (.004) among the Chamorros. The frequencies of the genes for color blindness and Xg^a are .033 and .65, respectively.

Part B included: Yes

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Plato, C. C.; Cruz, M. T. and Kurland, L. T.: Frequency of Glucose-6-Phosphate Dehydrogenase Deficiency, Red-Green Color Blindness and Xg^a Blood Group Among the Chamorros. Nature 202:728 (1964).

Honors and Awards relating to this project: None

Serial No. NDB (CF) - 62 E 964
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Electromyographic studies on ALS and PD

Principal Investigators: C. Van Nuis
T. S. Elizan

Other Investigators: B. M. Abrams
L. T. Kurland

Man Years

Total:	1/16
Professional:	1/16
Other:	0

Project Description:

An electromyographic study was completed on 41 patients: 25 with ALS, 12 with PD, and 4 cases of PD-ALS.

On the basis of the patients examined, it was found that the electromyographic findings among the ALS patients were indistinguishable from the findings among ALS patients reported elsewhere. The limited number of ALS-PD patients available for study again showed changes typical of anterior horn cell disease.

The group of PD patients studied revealed in a limited number of instances abnormalities consistent with lower motor neuron disease, either of peripheral or central type, without clinical lower motor neuron features.

The analysis of the above data has been included in the neurological paper by Dr. Elizan (see Serial No. NDB (CF) - 54 E 103, part II).

Part B included: No

Serial No. NDB (CF) - 63 E 1090
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Pedigree studies in Umatac

Principal Investigator: C. C. Plato

Other Investigator: M. T. Cruz

Man Years

Total:	5/8
Professional:	3/8
Other:	1/4

Project Description:

Umatac is a small village in southern Guam. It has the highest prevalence of ALS/PD on that island.

Several pedigrees collected earlier from this village have suggested that ALS/PD is a genetically controlled disease transmitted through a single dominant gene with incomplete penetrance. This, however, need not be the case. In a village as small and isolated as this one, with a presumably high degree of inbreeding, chance alone might be responsible for the high prevalence of familial occurrences.

The objectives of the present study are:

1. to trace and construct pedigrees for all the ALS/PD patients in the village;

2. to estimate the degree of consanguinity in this village; and

3. to evaluate the role of inheritance regarding the high prevalence of ALS/PD in this village.

The first phase of this project has been completed. Almost 97% of the village population was formed into a single pedigree which we were able to trace back for seven generations.

We are presently engaged in the estimation of the inbreeding coefficient and the examination of the role played by genetic inheritance in the etiology of ALS/PD.

Part B included: No

Serial No. NDB (CF) - 63 E 1091
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Serological and hematological investigations among the Trukese in Micronesia.

Principal Investigator: C. C. Plato

Other Investigator: M. T. Cruz

Man Years

Total:	3/8
Professional:	1/8
Other:	1/4

Project Description:

Concurrent with the clinical neurological examinations blood specimens were collected from three hundred and sixty Carolinians from the Truk and Yap districts. These specimens were typed for ABO, Rh, MNS, Kell, P, JK^b, and Kp^a systems. The plasma was used for haptoglobin determinations. With the hemolysater we carried out electrophoretic assays for detection of abnormal hemoglobins.

The objectives of this project were:

1. to detect possible associations between ALS/PD and the standard genetic markers (blood groups);

2. to estimate the genetic relationships between the various isolates under study;

3. to compare the blood group gene frequencies of the various areas of Truk and Yap in an effort to determine the origin of the Carolinians who have emigrated to Saipan; and

4. to detect, if possible, abnormal hemoglobins in this area.

The laboratory procedures have been completed, the data analyzed, and the calculations completed. A report is presently being prepared.

This project was formerly titled "Blood group frequencies of the Carolinians of Truk and Eastern Yap."

This project in its present form incorporates a former project titled "Hemoglobin and haptoglobin studies on the various Micronesian populations," Serial No. NDB (CF) - 62 E 963.

Part B included: No

Serial No. NDB (CF) - 63 E 1092
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Dermatoglyphic evaluation of the Chamorros and Carolinians.

Principal Investigators: C. C. Plato
J. Niswander
National Institute of Dental Research

Cooperating Unit: National Institute of Dental Research

Man Years

Total:	1/16
Professional:	1/16
Other:	0

Project Description:

This project was undertaken for two reasons:

1. to correlate fingerprint patterns with the neurological diseases found in these populations; and

2. to define the dermatoglyphics of these populations and compare them to those of other isolates.

Dermatoglyphics, like blood groups, are widely used as genetic markers as well as means of evaluating the relationship of genetic isolates. Since dermatoglyphics (finger as well as palm prints) are controlled by more than one pair of genes, their distributions are less vulnerable to genetic drift and isolation than the blood groups which are controlled by single genes.

The objectives of the project in its present form are:

1. to obtain additional genetic information regarding the Trukese and to use it, along with blood group data, to estimate the relationships between the various isolates in this geographic area; and

2. to determine whether isolates with common high values of blood sugar and uric acid also have similar dermatoglyphic patterns. From earlier studies it was found that the people of Micronesia have very high values of uric acid and blood sugar. These same high values were found in other populations including South American Indians. Since the dermatoglyphics of these isolates are available this comparison was deemed valuable.

Finger prints as well as palm prints from seventy Trukese were collected and are presently being evaluated.

Part B included: No

Serial No. NDB (CF) - 63 E 1093
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Blood group investigations on the Carolinians and Chamorros of Saipan

Principal Investigator: C. C. Plato

Other Investigators: D. L. Rucknagel
University of Michigan
L. T. Kurland

Cooperating Unit: University of Michigan , Ann Arbor, Michigan

Man Years
Total: 1/8
Professional: 1/8
Other: 0

Project Description:

Amyotrophic lateral sclerosis is found to be of high prevalence among both ethnic groups (Chamorro and Carolinian) residing in Saipan. This finding of high prevalence among two populations residing and working side by side gave more importance to the question of nature vs. nurture in regard to the etiology of ALS. It became of interest to investigate whether the two groups were genetically related; or whether the common high prevalence of ALS was due to environmental factors shared by both ethnic elements, or due to interaction of both heredity and environment.

The blood group investigations undertaken in this project were intended to supply evidence for or against genetic relationsip between these two groups of Saipan. In all 143 Chamorros and 146 Carolinians were included in this study. Each one was typed for the following systems: ABO (including A_1, A_2 and A_x), MNS, M^g, Rh (five antigens), P, V^w, Wr^a, Kell (including Kp^{ax} and Kp^b), Fy^a and JK^b. All C positive individuals were further tested for C^w and all D positive were investigated for D^u. The results gave evidence that the Carolinians and Chamorros of Saipan are genetically different.

A report is being prepared for publication.

Part B included: No

Serial No. NDB (CF) - 63 E 1094
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Protein electrophoresis of CSF and serum of ALS-PD cases and controls of Guam.

Principal Investigator: A. Lowenthal
Institut Bunge, Belgium

Other Investigators: T. S. Elizan
C. Van Nuis
J. M. Torres
L. T. Kurland

Cooperating Unit: Institut Bunge, Belgium

Man Years
Total: 1/16
Professional: 1/16
Other: 0

Project Description:

The proteins of CSF and serum of ALS-PD cases and controls have been studied electrophoretically in agar by a technique which had been perfected by Wieme, and which makes it possible to demonstrate approximately 20 different protein fractions. 43 ALS sera, 33 PD sera, 35 control sera, 9 ALS spinal fluids, 9 PD spinal fluids and 3 control spinal fluids were sent for analysis.

Except for the nonspecific high values of alpha-2 globulin and gamma globulin fractions in several cases, and a few changes in LDH activity and in various isoenzymes (the latter two being affected by age and storage), the results are within normal limits.

Part B included: No

Serial No. NDB (CF) - 63 E 1095
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Virus isolation and antibody studies in CSF and serum of ALS cases of Guam.

Principal Investigator: J. L. Sever
Perinatal Research Branch, NINDB

Other Investigators: T. S. Elizan
C. Van Nuis
J. M. Torres
M. T. Cruz
L. T. Kurland

Cooperating Unit: Perinatal Research Branch, NINDB

Man Years

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

The infectious (viral) etiology of ALS-PD has not been sufficiently and systematically studied. Twenty-four CSF specimens and 59 serum specimens from ALS-PD cases of Guam, and 25 control sera have been sent to the NIH laboratory for virus isolation and antibody studies. Serum antibody levels were determined for each of the following viruses: Adenovirus, Coxsackie A-2, A-4, A-8, A-9, B-1, B-2, B-3, B-4, B-5, B-6, Eastern Equine Encephalitis, Echo 6, 9, 10, 11, 16, 20, Flu A, B, C, Herpes Simplex, LCM, Mumps, Para 1, 2, 3, Polio I, II, III, Psittacosis, Respiratory Syncytial, Rubeola, Salivary Gland Virus, St. Louis Encephalitis, Varicella, Western Equine Encephalitis and Japanese B Encephalitis.

In January 1964, the complement fixation tests (serum) for Polio I, II, and III antibody for the above patients, were reported. There was one patient with antibody to Polio I, one with antibody to Polio II, and 11 with antibody to Polio III. Neutralization tests were also conducted for Polio virus

types I, II, and III, and for several arbor viruses.

Statistical analysis failed to demonstrate any significant difference in the frequency of antibody between the cases and their matched controls.

Part B included: No

Serial No. NDB(CF) - 63 E 1096
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Virus isolation studies in brain tissues of ALS-PD patients of Guam.

Principal Investigators: C. Gajdusek
Collaborative & Field Research, NINDB
C. J. Gibbs
Collaborative & Field Research, NINDB

Other Investigators: T. S. Elizan
C. Van Nuis
B. M. Abrams
L. T. Kurland

Cooperating Unit: Collaborative & Field Research, NINDB

Man Years
Total: 1/16
Professional: 1/16
Other: 0

Project Description:

Monkeys have been inoculated intracerebrally with CNS emulsion from patients with ALS, by Dr. Zilber of Moscow, who has reported the development of muscular weakness and wasting in his animals one to two years following such inoculations. In an effort to follow through on Zilber's work, brain specimens of three Guam ALS patients and of two PD patients have been removed aseptically within the first two to three hours after death, quickly preserved in liquid nitrogen and sent to the NIH Patuxent Laboratory for virus inoculation studies in 1963. Corresponding sites of brain tissue were sent to Dr. Zilber's laboratory in Moscow for identical inoculation studies, and to Dr. Hirano's laboratory in New York for histological examination.

No significant report is available yet at this time (April, 1965).

Part B included: No

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Clinical aspects of diabetes mellitus in Guam.

Principal Investigator: R. L. Need

Other Investigator: T. S. Ellis

Man Years

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

The clinical aspects of diabetes mellitus in Guam were studied. Of particular interest was the previously reported apparent absence of juvenile diabetes and of ketoacidosis in Chamorros. The study encompassed known diabetics seen at the Guam Memorial Hospital and in the NINDB clinics during 1963-1964 and included glucose tolerance tests, past medical history, physical findings and family histories.

Only one case of juvenile diabetes was seen and no instance of diabetic acidosis was observed. Moreover, very few cases of diabetic neuropathy were seen in these Chamorro patients.

Part B included: No

Serial No. NDB (CF) - 63 E 1103
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Neurological diseases other than ALS-PD on Guam.

Principal Investigators: T. S. Elizan
K. M. Chen
Y. Yase

Other Investigators: R. L. Need
B. M. Abrams
A. Hirano
L. T. Kurland

Man Years

Total:	1/4
Professional:	3/16
Other:	1/16

Project Description:

The NINDB has occupied a unique position on Guam since 1956. It is the only neurological consultation service available to the Chamorro, Filipino, and Caucasian population on Guam and sees most neurological patients at the two hospital facilities - Guam Memorial Hospital and Navy Hospital. Therefore, most cases of significant neurological disease are eventually brought to its staff's attention.

Dr. Hirano classified 200 neurological patients seen from September 1, 1959 to June, 1960 without regard to dividing Chamorros from non-Chamorros.

The present study reanalyzes all Chamorro non-ALS, PD or suspect cases as to neurological entities. If the patient presents any question of diagnosis and is still alive, he will be reexamined and have relevant diagnostic studies. All cases of rare neurological disease still living will be reexamined.

If, in the 300-odd cases available for study by case history, any disease entity appears to obtain in greater numbers than would be expected in previous statistical experience,

appropriate steps will be taken to assemble material and gather statistical data about the appearance on Guam with a further view to investigating its etiology and pathogenesis.

Respectfully submitted,

Serial No. NDB (CF) - 63 E 1104
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Measles and other virus antibody in patients with multiple sclerosis

Principal Investigators: D. M. Reed
J. L. Sever
Perinatal Research Branch, NINDB

Other Investigators: J. Kurtzke
Mount Alto Veterans Administration Hospital
L. T. Kurland

Cooperating Units: Perinatal Research Branch. NINDB
Mount Alto Veterans Administration Hospital
Washington, D. C.

Man Years
Total: 1/16
Professional: 0
Other: 1/16

Project Description:

This study was prompted by the need for independent verification of a report by Adams and Imagawa indicating a relationship between measles antibody and multiple sclerosis.

Accordingly, the blood and spinal fluid of 35 multiple sclerosis patients and 35 comparison subjects were tested for neutralization, hemagglutination inhibition and complement fixation (CF) antibody to measles virus and CF antibody to nine other common viruses.

The results showed no significant difference between the two groups in regard to measles antibody in the spinal fluid or to other virus antibody in the spinal fluid or blood. The most specific or sensitive tests for measles antibody in the blood did not correlate with multiple sclerosis; however, the relatively crude CF test did show a significant positive relationship. The explanation for this last finding is not readily apparent, but it is not believed to indicate any causative role for measles virus in multiple sclerosis.

Part B included: Yes

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Reed, D.; Sever, J.; Kurtzke, J. and Kurland, L.: Measles and other virus antibody in patients with multiple sclerosis. Archives of Neurology 10:402-410 (1964).

Honors and Awards relating to this project: None

Serial No. NDB (CF) - 63 E 1106
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Screening of edible and medicinal legumes for neurotoxic constituents

Principal Investigator: M. G. Whiting (Coordinator)

Other Investigators: F. Strong
University of Wisconsin
E. A. Bell
King's College
F. R. Fosberg
National Academy of Sciences
Q. Jones
USDA

Cooperating Units: University of Wisconsin , Madison, Wisconsin
King's College, London, England
National Academy of Sciences, Washington, D. C.
U. S. Department of Agriculture, Beltsville, Md.

Man Years
Total: 1/16
Professional: 1/32
Other: 1/32

Project Description:

The objective of this research is to assess the neurotoxic components of a variety of edible and medicinal legumes from focal areas of high prevalence of neurological disease. Samples of legumes used for food or medicine have been collected, identified and tested. (See TABLE, next page)

An additional supply of "nagi" (Podocarpus nagi Makino) was requested by Dr. Strong for further study. The plant is used as a medicinal tea in Mitogawa and, perhaps, in other areas of Japan. Dr. Strong suggests that it may contain either β-aminoproprionitrile or β-cyalanine.

To date Dr. Bell has not identified nitriles or oxalyl detivatives or alpha-gamma-aminobutyric acid in any sample, but he has found high concentrations of an acidic peptide in seeds from ten different plants. Additional material for further testing has been sent to him.

	Country of Origin		
	Guam	Japan	
Voucher specimens	40[1]	36	Identified by R. Fosberg
Seeds	0	46	Identified by Q. Jones
Seeds	0	37	Tested by F. Strong[2]
Seeds	0	46	Tested by E. Bell[2]

[1] Twelve used for food, 14 for medicine, and four for both food and medicine

[2] Tested for lathyrogenic components

Supplies of seeds sufficient for testing have been requested from the Department of Agriculture in Guam.

Part B included: No

Serial No. NDB (CF) - 63 E 1107
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Food, medicinal, and toxic plants of Mitogawa, Japan.

Principal Investigator: M. G. Whiting

Other Investigators: A. Ko
Shingu, Japan
H. Matsushita
Wakayama, Japan
F. R. Fosberg
National Academy of Sciences

Cooperating Unit: National Academy of Sciences, Washington, D. C.

Man Years

Total:	1/4
Professional:	1/8
Other:	1/8

Project Description:

The objective of this research is to provide a working botanical reference to commonly ingested plants from the Mitogawa area of Japan.

Information has been collected regarding the utilization of local plants for medicine and food. Many leguminous plants are used as medicinal teas in the Mitogawa area and several specimens of suspected toxicity are included in both the food and medicinal lists. Botanists in the area have cooperated during the past year by collecting additional voucher specimens as requested to supplement the collection made by Dr. Whiting in 1963-1964. Identification has now been made, under the direction of Dr. Fosberg, of a total of 130 specimens. Plants will now be selected from the list for further field and laboratory study.

It is expected that leads will be obtained which will contribute to the study of environmental factors in relation to the high frequency of neurological diseases in the Mitogawa area.

Part B included: No

Serial No. NDB (CF) - 63 E 1108
1. Collaborative and Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Study of Thiaminase and Heat Labile Anti-B_1 Metabolite in "Zenmai" (Osmunda japonica Thunb.)

Principal Investigator: M. G. Whiting (Coordinator)

Other Investigator: H. Matsumoto
University of Hawaii

Cooperating Unit: Agricultural Biochemistry Department, University of Hawaii, Honolulu, Hawaii

Man Years

Total:	1/16
Professional:	1/32
Other:	1/32

Project Description:

Osmunda japonica Thunb. is one of several wild food plants, commonly used by the residents of Mitogawa, Japan, which is reputed by local physicians to contain an anti-B_1 metabolite in sufficient quantities to have a deleterious effect upon the health of local residents.

Results of animal feeding experiments with commercial zen mai purchased in Hawaii and conducted under the direction of Dr. Matsumoto indicate that boiling the plant in water removes its growth-inhibiting properties, and that the growth-inhibiting effect is due to a thiamine deficiency. These results are substantiated by evidence already present in the literature.

Two variables require further study: 1) fungal growths were visible on the samples sent to Dr. Matsumoto from Mitogawa while the samples he purchased in Hawaii were packaged with an anti-fungal and contained no visible fungal contamination. Further work is indicated on the possible toxicity of fungi commonly found on zen mai collected and processed in Mitogawa,

and 2) it was not possible to make a positive identification of the dried samples of zen mai purchased in Hawaii. Additional studies should be conducted using plant material obtained from Mitogawa.

Part B included: No

Serial No. NDB (CF) - 63 E 1109
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Study of toxic fungi in cycad and other plant material used for human or animal food in various areas of the world.

Principal Investigator: M. G. Whiting

Other Investigators: J. Forgacs
Good Samaritan Hospital
W. T. Carll
Bluff Run Farm

Cooperating Units: Good Samaritan Hospital, Suffern, New York
Bluff Run Farm, Hopkins, South Carolina

Man Years
Total: 3/8
Professional: 1/4
Other: 1/8

Project Description:

This research was designed to determine the presence of toxic, particularly neurotoxic, fungi in native plant material used for food or medicine by Man or animals in such areas as Guam and the Kii Pen insula, Japan, where neurological diseases occur with high incidence. Special attention was given to cycad plant material.

Samples examined for toxic fungi included the following:

Area of Origin	From Cycads	From other foods & chews	Total
Japan	11	15	26
Guam	49	8	57
Florida	9	0	9
Dominican Republic	4	0	4

To date, eight of the isolates have been found to produce acute toxic effects when given orally to mice. As a result of these studies, Dr. Forgacs especially recommends additional research on the following food items and fungi:

Foods	Area of origin	Fungus isolated
Miso soup powder (made with starch from Cycas revoluta)	Japan	Aspergillus oryzae
Seeds and flour from C. Circinalis	Guam	Aspergillus niger Aspergillus flavus Link Phoma spp. curvularia lunata
Husks of seeds of C. Circinalis	Guam	Penicillium purpurogenum (This genus contains highly toxic species known to kill quickly without marked blood changes.)
Leaves and tubers of Zamia debilis L. F. in Ait.	Dominican Republic	Mycosphaerella spp. (This genus contains several species known to be pathogenic, Ex. tulasni)

This work was performed under a Professional and Technical Services Contract, terminated on 30 April 1964. The final report was received on 18 December 1964.

Part B included: No

Serial No. NDB (CF) - 63 E 1110
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Preliminary study of plants from the island of Guam: Food, Medicinal, and Toxic.

Principal Investigator: M. G. Whiting

Other Investigator: F. R. Fosberg
National Academy of Sciences

Cooperating Unit: National Academy of Sciences , Washington, D. C.

Man Years

Total:	1/4
Professional:	1/8
Other:	1/8

Project Description:

The objective in this research is to provide a basic botanical reference for use by research workers interested in environmental factors in relation to disease patterns in Guam.

Data on food and medicinal use of plants from Guam is being prepared by Dr. Whiting for publication. Material from four plant families (Cycadaceae, Gramineae, Pandanaceae, and Convolvulaeae) has been forwarded to Dr. Fosberg for inclusion in his progress report on a Flora of Guam, in preparation under a Professional and Technical Services Contract (Project Officer, Dr. William F. Caveness).

Part B included: No

Serial No. NDB (CF) - 63 E 1111
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Neurotoxic effects from consumption of cycad plant material.

Principal Investigator: M. G. Whiting (Coordinator)

Other Investigators:
J. L. Anderson
Department of Agriculture, Stock & Fisheries
W. K. Engel
Medical Neurology Branch, NINDB
A. Frederickson
Mason Research Institute
C. Gallagher
University of Sydney
W. J. Hadlow
Rocky Mountain Laboratory, NIAMD
W. T. K. Hall
Animal Research Institute
J. A. Henderson
Washington State University
J. Innes
Bionetics Research Lab., Inc.
M. Mason
Mason Research Institute
C. Hoch-Ligeti
Veterans Administration
R. Zinn and A. Allen
Laboratory Aids Branch, DRS

Cooperating Units:
Division of Animal Industry, Department of Agriculture, Stock & Fisheries, Territory of Papua and New Guinea, Australia
Medical Neurology Branch, NINDB
McMaster Animal Health Laboratory, University of Sydney, Sydney, Australia
Rocky Mountain Laboratory, NIAMD
Animal Research Institute, Yeerongpilly, Brisbane, Queensland, Australia
College of Veterinary Medicine, Washington State University, Pullman, Washington

Serial No. NDB (CF) - 63 E 1111

South Puerto Rico Sugar Company Trading Corporation, New York, New York
Bionetics Research Lab., Inc., Falls Church Virginia
Mason Research Institute, Worcester, Massachusetts
Veterans Administration, Martinsburg, West Virginia
Poolesville Animal Farm, DRS

Man Years

Total:	1/4
Professional	1/16
Other	3/16

Project Description:

This research is designed to isolate and identify the component of cycad plant material responsible for the reported neurotoxic effects on cattle.

To coordinate investigations of this project in Australia and in the Caribbean, a meeting was held on 26 October 1964 when both Dr. Gallagher from Sydney and Dr. Henderson, livestock consultant to the South Puerto Rico Sugar Company Trading Corporation in the Dominican Republic, could be present (Mimeograph report on file). Dr. Henderson reported that several hundred head of cattle from a herd of 40,000 are lost each year with a lameness called "derringue" attributed to the ingestion of cycad leaves common in local pastures. Dr. Gallagher indicated his interest and willingness to follow developments both in Queensland (with Dr. Hall) and in Papua (with Dr. Anderson).

Material from afflicted animals sent to NIH in 1964 by Dr. Anderson and prepared by Dr. Hadlow has been examined by Drs. Allen, Hadlow and Innes. No lesions were found. Material prepared by Hall and sent to NIH was examined by Dr. Innes who found no lesions to account for the lameness observed in the animals. Dr. Anderson is now sending autopsy material from afflicted animals to Dr. Gallagher in Sydney.

In December, Dr. Whiting visited the Dominican Republic and brought back a film showing afflicted animals, cycad plants for identification and for the study of fungi, and cycad leaves for animal experimentation. Considerable starch from the local cycad plant is prepared and consumed by the native population.

The cycad plant species common in the Dominican Republic has been identified by Dr. Fosberg as Zamia debilis L.F. in Ait. It grows profusely in pasture land.

Dr. Forgacs found that the fungus Mycosphaerella spp. predominates on both leaves and tuber of Zamia. This genus is known to contain pathogenic species. By feeding various levels of leaves of Zamia debilis L.F. in Ait. to rats, Dr. Hoch-Ligeti has determined lethal dosage and noted evidence of acute toxicity. Experiments will be continued to observe effects of long-term feeding.

Arrangements were made with the South Puerto Rico Sugar Company for Drs. Mason and Frederickson to go to the Dominican Republic in February 1965. During a ten-day visit, they observed and photographed 30 or more afflicted animals, of which they slaughtered six. Dr. Mason presented a preliminary report of his trip at the Fourth Conference on the Toxicity of Cycads on 15 April 1965. Specimens are now under study by Drs. Mason, Frederickson and Innes. Suggestions under discussion for future research plans in the Dominican Republic include an epidemiological study of cancer and neurological disease in that country, animal feeding trials, analyses of plants and soil, and further clinical and pathological study of the cattle ailment, derriengue.

Part B included: No

Serial No. NDB (CF) - 63 E 1112
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Chemistry of the cycad.

Principal Investigator: M. G. Whiting (Coordinator)

Other Investigators:
H. Matsumoto
University of Hawaii
T. Nagahama
University of Hawaii
H. Larson
University of Hawaii
N. V. Riggs
University of New England
B. H. Korsch
University of New England
P. N. Magee
Medical Research Council Laboratory
R. C. Shank
Massachusetts Institute of Technology

Cooperating Units:
Agricultural Biochemistry Department, University of Hawaii, Honolulu, Hawaii
Department of Organic Chemistry, University of New England, Armidale, N.S.W., Australia
Toxicology Research Unit, Medical Research Council Laboratory, Carshalton, Surrey, England
Massachusetts Institute of Technology, Cambridge, Massachusetts

Man Years

Total:	1/16
Professional:	1/32
Other:	1/32

Project Description:

Chemical research on cycads has been directed toward isolation, identification and synthesis of toxic components from portions of the plant which are used for food.

Drs. Matsumoto, Nagahama, and Larson have been successful in synthesizing the acetate of methylazoxymethanol (MAM acetate) which is the aglycone and toxic component of the glycosides found in various species of cycads. (See Biochemical Journal April, 1965 and report of the Fourth Conference on the Toxicity of Cycad, NIH, 15 April 1965).

Plans are far advanced for commercial production of MAM acetate. New experiments can now be designed to take advantage of a labelled compound which will soon be available.

A doctoral thesis, Studies on Neocycasins by T. Nagahama written in Japanese is now available as NIH Translation 12-11-64.

Part B included: No

Serial No. NDB (CF) - 63 E 1113
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Effects on animals following administration of cycad plant material and of toxic compounds isolated from cycads.

Principal Investigator: M. G. Whiting (Coordinator)

Other Investigators:
G. Laqueur
Laboratory of Experimental Pathology, NIAMD
J. Williams
Laboratory of Nutrition & Endocrinology, NIAMD
M. Rechcigl
Laboratory of Biochemistry, NCI
M. Spatz
Laboratory of Experimental Pathology, NIAMD
D. Smith
Laboratory of Experimental Pathology, NIAMD
M. Kelly
Laboratory of Chemical Pharmacology, NCI
R. O'Gara
Laboratory of Pathology, NCI
R. Zinn
Laboratory Aids Branch, DRS
C. Hoch-Ligeti
Veterans Administration
P. Newberne
Massachusetts Institute of Technology
O. Mickelsen
Michigan State University
M. Yang
Michigan State University
E. Campbell
Michigan State University
G. Mugera
Michigan State University
H. Matsumoto
University of Hawaii
H. Teas
University of Georgia

Cooperating Units: Laboratory of Experimental Pathology, NIAMD
Laboratory of Biochemistry, NCI
Laboratory of Chemical Pharmacology, NCI
Laboratory of Pathology, NCI
Poolesville Farm, Laboratory Aids Branch, DRS
Veterans Administration, Martinsburg, West Virginia
Massachusetts Institute of Technology, Cambridge, Massachusetts
Department of Foods and Nutrition, College of Home Economics, Michigan State University
Agricultural Biochemistry Department, University of Hawaii, Honolulu, Hawaii
University of Georgia, Athens, Georgia
Laboratory of Nutrition & Endocrinology, NIAMD

Man Years:

Total:	1/16
Professional:	1/32
Other:	1/32

Project Description:

On-going experiments continue to contribute to our understanding of the effects on several species of animals with the administration of portions of cycad plant material. Acute and long-term manifestations are being studied. Details are available in reports of the Third Conference on the Toxicity of Cycads (Federation Proceedings 23:6, Part I, 1335-1387, 1964) and the Fourth Conference on the Toxicity of Cycads, 15 April 1965 (in preparation). See also report of G. Laqueur, NIAMD.

Part B included: No

Serial No. NDB (CF) - 63 E 1114
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS -NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Effects of fresh cycad kernel meal on open wounds

Principal Investigator: R. O'Gara
NCI

Other Investigators: M. G. Whiting
J. M. Brown
NCI

Cooperating Unit: NCI

Man Years
Total: 1/16
Professional: 1/32
Other: 1/32

Project Description:

Reports are available from Indonesia, Guam, Manus Island, Dominican Republic, Philippines, Indochina and India that sap from fresh cycad kernels is used to promote rapid healing of skin ulcers and open wounds. To simulate this folk use of a native medicine, the effects of an aqueous extract of fresh cycad kernel on artificial skin ulcers in mice were studied.

In a preliminary experiment in which 40 mice were used, the ulcers healed quickly but upon histological examination, skin at the site of the wound occasionally showed a group of atypical epithelial cells. After a delay of many months, hepatoma, adenomas of kidney, a multilocular hepatic cyst, and a hemangioendothelioma of the liver were among the pathological findings.

The experiments will be repeated with a larger number of animals.

Part B included: Yes

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

O'Gara, R.; Brown, J. M. and Whiting, M. G.: Induction of hepatic and renal tumors by the topical application of aqueous extract of cycad nut (C. circ.) to artificial skin ulcers. Federation Proceedings 23:6, Part 1 (November-December 1964)

Honors and Awards relating to this project: None

Serial No. NDB (CF) - 63 E 1117
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Epidemiological studies of multiple sclerosis in New Orleans, Louisiana

Principal Investigator: A. Stazio

Other Investigators:
L. T. Kurland
R. Paddison
Louisiana State University
R. Paterson
Tulane University
J. Paterson
Tulane University
J. Moosey
Louisiana State University
T. Soniat
Ochsner Clinic

Cooperating Units: Louisiana State University School of Medicine
Tulane University, New Orleans, Louisiana
Ochsner Clinic, New Orleans, Louisiana

Man Years
Total: 1
Professional: 1
Other: 0

Project Description:

A follow-up of previous population studies of multiple sclerosis conducted in New Orleans in 1949 and 1951 by Kurland and Westlund, and a re-survey of multiple sclerosis prevalence and incidence in New Orleans are underway. The objectives of the study are: (1) to evaluate the accuracy of the 1949 and 1951 case selections; (2) to provide information on survivorship patterns and the natural history of multiple sclerosis; (3) to clarify certain aspects of the distribution of the disease in New Orleans; and (4) to determine prevalence and incidence trends over the past 20 years.

An additional objective of the study is to detect possible environmental factors responsible for the disease by comparing the distribution pattern and the characteristics of the disease between New Orleans and Winnipeg, Canada, where a similar population study of multiple sclerosis has just been completed.

Part B Included: No

Serial No. NDB (CF) - 63 E 1118
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Epidemiological studies of multiple sclerosis in Winnipeg, Manitoba, Canada

Principal Investigator: A. Stazio

Other Investigators: L. T. Kurland
L. G. Bell
University of Manitoba
M. G. Saunders
University of Manitoba
M. E. McGuire
Winnipeg General Hospital
R. T. Ross
University of Manitoba

Cooperating Units: University of Manitoba School of Medicine, Canada
Winnipeg General Hospital , Manitoba, Canada

Man Years
Total: 1/8
Professional: 1/16
Other: 1/16

Project Description:

A follow-up and reevaluation of the 1951 multiple sclerosis survey of Winnipeg, Canada, by Westlund and Kurland, has shown that approximately one third of the 1951 multiple sclerosis population in Winnipeg had been missed in the original survey, while approximately 25 percent of the cases reported in 1951 had been misdiagnosed. The revised prevalence rate of probable patients was 36.2 per 100,000. This figure is close to the original survey estimate of 39.6; however, there were appreciable discrepancies in the individuals comprising the two groups of patients.

Mortality statistics based only on death certificates would have disclosed only 60 percent of the patients known to have expired by the end of the follow-up study.

The average duration of the disease for all patients was estimated to be not less than 21 years.

The average annual incidence rate per 100,000 population was 1.5. The data also suggests that the incidence of multiple sclerosis in Winnipeg has remained unchanged for the past 20 years.

Part B included: Yes

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Stazio, A.; Kurland, L. T.; Bell, L. G.; Saunders, M. G. and Rogot, E.: Multiple sclerosis in Winnipeg, Manitoba. Methodological considerations of epidemiologic survey. Journal of Chronic Diseases. 17:415-438 (1964).

Kurland, L. T.; Stazio, A. and Reed, D. M.: An appraisal of population studies of multiple sclerosis. Annals of the New York Academy of Sciences 122:520-541 (1965).

Honors and Awards relating to this project: None

Serial No. NDB (CF) - 63 E 1119
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Investigation of an outbreak of keratoconjunctivitis in La Paz, Bolivia.

Principal Investigators: J. T. Schwartz
S. Foster
Public Health Hospital

Other Investigators: P. Thygeson
University of California
E. Jawetz
University of California

Cooperating Units: Public Health Hospital, Phoenix, Arizona
Francis I. Proctor Foundation, University of California Medical Center, San Francisco, California
Department of Microbiology, San Francisco Medical Center, University of California, San Francisco, California

Man Years

Total:	1/16
Professional:	1/32
Other:	1/32

Project Description:

This project was undertaken in consultation with the Medical Program Division of the Peace Corps. A diffuse outbreak of keratoconjunctivitis had developed among the population of La Paz, and the attack rate among North Americans living in this area was high. 83 North Americans were examined grossly and also by means of fluorescein biomicroscopy for the presence of external eye disease. Mild (grade 1 +) conjunctival follicles were present among approximately one-half, and superficial punctate staining of the cornea was present in approximately one-third of those examined.

Analysis of patient histories revealed no identifiable pattern of transmission. The ocular findings were not consistent

with any accepted pattern of viral keratoconjunctivitis. To further investigate the possibility of a viral etiology, viral cultures, conjunctival smears and blood sera have been examined by collaborators at the University of California. Laboratory tests failed to support the possibility of viral etiology.

In this outbreak, a causal role of environmental factors has not been ruled out. Decreased partial pressure of atmospheric oxygen, and increased ultraviolet exposure at this altitude (approximately 13,000 feet) may hold some importance in the etiology of these mild cases of keratoconjunctivitis.

Part B included: No

Serial No. NDB (CF) - 63 E 1123
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: An evaluation of the comparability among a sample of certified Schiotz tonometers.

Principal Investigator: J. T. Schwartz

Other Investigator: S. S. Ambler

Cooperating Unit: United States Soldier's Home, Washington, D.C.

Man Years

Total:	1/32
Professional:	1/32
Other:	0

Project Description:

A study to evaluate the performance of a sample of certified Schiotz tonometers, in order to: 1) help clarify the possible influence of tonometer variability on the outcome of epidemiologic field studies, and 2) select for future field use, a small number of tonometers known to have been capable of agreement.

The need for a similar prevailing level of ocular tension among examined eyes has been an obstacle to the successful biologic comparison of identation tonometers. This study was designed to minimize the need for similar prevailing tensions among examined subjects by collecting data with paired instruments on each eye. Potential bias was neutralized by appropriate randomization procedures.

Data analysis revealed a maximum difference of approximately one half scale unit between paired tonometers with the 7.5 gram weight. A difference of this magnitude would be expected to materially alter the outcome of a tonometry survey.

Part A included: Yes

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Schwartz, J. Theodore and Ambler, Sydney S.: Comparability of a sample of certified Schiotz tonometers; with an evaluation of the potential influence of tonometer variability on the outcome of epidemiologic field studies. Archives of Ophthalmology 72:672-678 (1964).

Honors and Awards relating to this project: None

Serial No. NDB (CF) - 63 E 1124
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Evaluation of the kuru genetic hypothesis

Principal Investigator: G. R. Williams

Other Investigators: A. Fischer
Tulane University
J. L. Fischer
Tulane University
L. T. Kurland

Cooperating Unit: Tulane University, New Orleans, Louisiana

Man Years:

Total:	1/16
Professional:	0
Other:	1/16

Project Description:

The genetic hypothesis of the cause of kuru was evaluated by mathematical and statistical techniques. It was demonstrated that the hypothesis in its present form does not adequately account for the unique age and sex distribution of this disease.

Part B included: Yes

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Williams, G. R.; Fischer, A.; Fischer, J. L. and Kurland, L. T.: An evaluation of the kuru genetic hypothesis. Journal de Genetique Humaine 13:11-21 (1964).

Honors and Awards relating to this project: None

Serial No. NDB (CF) - 63 E 1130
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Fine Structure of Cerebral Fluid Accumulation. Part III. Extracellular Spread of Cryptococcal Polysaccharides in the Acute State.

Principal Investigator: A. Hirano

Other Investigators: H. M. Zimmerman
Montefiore Hospital
S. Levine
St. Frances Hospital

Cooperating Units: Montefiore Hospital , New York, New York
St. Frances Hospital , New Jersey

Man Years

Total:	1/32
Professional:	0
Other:	1/32

Project Description:

Cryptococcal polysaccharide were implanted in the rat brain, and their selective spread in the white matter was studied by light and electron microscopy after the whole brain was fixed in situ by perfusion.

In the acute stage, electron-dense fluid accumulated mainly extracellularly in the area of distant spread. It extended selectively in the white matter between the myelinated nerve fibers, non-myelinated nerve fibers and glial cell processes.

Part B included: Yes

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Hirano, A.; Zimmerman, H. M. and Levine, S.: The fine structure of cerebral fluid accumulation. Part III. Extracellular spread of cryptococcal polysaccharides in the acute state. American Journal of Pathology 45:1-19 (1964).

Honors and Awards relating to this project: None

Serial No. NDB (CF) - 63 E 1131
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Fine Structure of Cerebral Fluid Accumulation. Part IV. On the Nature and Origin of Extracellular Fluids Following Cryptococcal Polysaccharide Implantation.

Principal Investigator: A. Hirano

Other Investigators: H. M. Zimmerman
Montefiore Hospital
S. Levine
St. Frances Hospital

Cooperating Units: Montefiore Hospital , New York, New York
St. Frances Hospital , New Jersey

Man Years

Total:	1/32
Professional:	0
Other:	1/32

Project Description:

Implants of cryptococcal polysaccharide in rat brain produced an accumulation of fluid which was distributed selectively in white matter. Electron microscopic identification of cryptococcal polysaccharide, and of polysaccharide-containing fluid, was made possible by the strong affinity of this material for metals. The fluid originated from the implant and occupied the extracellular space. In the early phase of fluid spread, and in areas close to the implant, the extracellular space contained a second type of fluid. The later was thought to be of hematogenous origin because of its morphology, staining reaction, proximity to vessels and relation to gaps in the perivascular ring of astrocytic foot processes. The two types of fluid were able to coexist for a brief time, contiguous but unmixed, despite the absence of any physical barrier between them.

Part B included: Yes

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Hirano, A.; Zimmerman, H. M. and Levine, S.: The fine structure of cerebral fluid accumulation. Part IV. On the nature and origin of extracellular fluids following cryptococcal polysaccharide implantation. American Journal of Pathology 45:195-207 (1964).

Honors and Awards relating to this project: None

Serial No. NDB (CF) - 64 E 1246
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Fine Structure of Cerebral Fluid Accumulation. Part V. Transfer of Fluid From Extracellular to Intracellular Compartments in Acute Phase of Cryptococcal Polysaccharide Lesions.

Principal Investigator: A. Hirano

Other Investigators: H. M. Zimmerman
Montefiore Hospital
S. Levine
New York Medical College Center for Chronic Disease

Cooperating Units: Montefiore Hospital , New York, New York
New York Medical College Center for Chronic Disease, New York, New York

Man Years
Total: 1/2
Professional: 3/8
Other: 1/8

Project Description:

Cryptococcal polysaccharide implanted into the rat forebrain spread into the white matter by way of the extracellular spaces. Relatively little material entered the cells during the acute phase of reaction. There were two different methods by which small amounts of extracellular fluid entered the intracellular compartment.

Direct Extension of the Material into Cytoplasm Through Ruptured Glial and Neuronal Plasma Membranes -- This type was observed exclusively in tissue surrounding the original implant. Intracellular material was diffusely distributed in the cytoplasm and presented the same texture and density as the extracellular fluid. The density decreased with passage of time, probably due to dilution.

Active Intake of Material by Phagocytosis -- A few macrophages appeared at the site of implantation as well as in the area of distant fluid spread. The material was engulfed by their infolding plasma membranes to form multiloculated intracytoplasmic inclusions which were denser than the extracellular material, possibly due to removal of water.

This electron microscopic study has made it possible to elucidate some of the dynamic processes involved in transfer of fluid from extracellular compartments in the brain.

Part B included: Yes

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Hirano, A.; Zimmerman, H. M. and Levine, S.: Fine Structure of Cerebral Fluid Accumulation. Part V. Tranfer of Fluid From Extracellular to Intracellular Compartments in Acute Phase of Cryptococcal Polysaccharide Lesions. Archives of Neurology 11:632-648 (1964).

Honors and Awards relating to this project: None

Serial No. NDB (CF) - 64 E 1247
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Fine Structure of Cerebral Fluid Accumulation. Part VI. Intracellular Accumulation of Fluid and Cryptococcal Polysaccharide in Oligodendroglia.

Principal Investigator: A. Hirano

Other Investigators: H. M. Zimmerman
Montefiore Hospital
S. Levine
New York Medical College Center for Chronic Disease

Cooperating Units: Montefiore Hospital, New York, New York
New York Medical College Center for Chronic Disease, New York, New York

Man Years
Total: 1/2
Professional: 3/8
Other: 1/8

Project Description:

Pellets of cryptococcal capsular polysaccharides mixed with graphite were implanted in the rat forebrain. The subacute and chronic phases of the experiment were studied with the aid of light and electron microscopy in brains fixed in toto by perfusion.

Polysaccharide-rich fluid spreads mainly in the extracellular spaces of white matter during the initial 48 hours. Three or four days after implantation there was a sudden and dramatic shift of fluid from extracellular to intracellular compartments. After one week there was no longer any fluid in intracellular spaces. Instead, the interfascicular oligodendroglia became distended by numerous vacuoles filled with polysaccharide-rich fluid. These ballooned cells persisted at least three months. This unique

reaction of oligodendroglia to certain polysaccharides may have some relationship to the classical alterations known as acute swelling and mucoid degeneration of oligodendroglia.

Part B included: Yes

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Hirano, A.; Zimmerman, H. M. and Levine, S.: The Fine Structure of Cerebral Fluid Accumulation. Part VI. Intracellular Accumulation of Fluid and Cryptococcal Polysaccharide in Oligodendroglia. Archives of Neurology 12:189-196 (1965).

Honors and Awards relating to this project: None

Serial No. NDB (CF) - 65 E 1248
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Fine Structure of Cerebral Fluid Accumulation. Part VII. Reactions of Astrocytes to Cryptococcal Polysaccharide Implantation.

Principal Investigator: A. Hirano

Other Investigators: H. M. Zimmerman
Montefiore Hospital
S. Levine
New York Medical College Center for Chronic Disease

Cooperating Units: Montefiore Hospital, New York, New York
New York Medical College Center for Chronic Disease , New York, New York

Man Years

Total:	1/2
Professional:	3/8
Other:	1/8

Project Description:

Implantation of cryptococcal polysaccharides in rat brain resulted in the spread of exogenous material with fluid into the distant white matter. Changes of fibrous astrocytes in the area of implantation and in distant white matter were studied with light and electron microscopy.

Clear appearing astrocytes were observed in the vicinity of the implant during the acute stage. A watery and voluminous expansion of cytoplasm was accompanied by many minute vesicular profiles and a considerable increase of glycogen-like granules. In the chronic stage, the increase of glial fibrils was the most conspicuous phenomena.

Direct invasion of polysaccharide-rich fluid into the astrocytes was observed through ruptured plasma membranes at the site of implant. Diffuse invasion by a fluid mass left

a large lake of polysaccharide within the affected astrocyte. In addition to diffuse infiltration of exogenous material, small cystic focal collections of fluid were observed in the affected astrocytes. The morphological texture of ingested material in these cells remained remarkably unchanged. Persistence of large amounts of intracellular polysaccharide was observed even 100 days after implantation.

Part B included: Yes

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Hirano, A.; Zimmerman, H. M. and Levine, S.: Fine Structure of Cerebral Fluid Accumulation. Part VII. Reaction of Astrocytes to Cryptococcal Polysaccharide Implantation. Journal of Neuropathology and Experimental Neurology (in press).

Honors and Awards relating to this project: None

Serial No. NDB (CF) - 65 E 1249
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Fine Structure of Cerebral Fluid Accumulation. Part VIII. Experimental Leukoencephalopathy Due to Implantation of Foreign Substances.

Principal Investigator: A. Hirano

Other Investigators: H. M. Zimmerman
Montefiore Hospital
S. Levine
New York Medical College Center for Chronic Disease

Cooperating Units: Montefiore Hospital, New York, New York
New York Medical College Center for Chronic Disease , New York, New York

Man Years

Total:	1/2
Professional:	3/8
Other:	1/8

Project Description:

Cryptococcal polysaccharides were implanted into the rat brain, and their selective spread in the white matter was studied with light and electron microscopy, after the whole brain was fixed in situ by perfusion.

The cryptococcal polysaccharides in fluid were observed as electron-dense reticular substance with uranyl acetate or lead hydroxide stains. It originated from the region of the implant and moved a considerable distance in the white matter by way of the extracellular space in the acute stage. The location of fluid changed considerably as the lesion aged. The fluid flow was reduced in 3 to 4 days, and the fluid was exclusively localized within the glial cells after one week.

In the early phase of fluid spread, and in areas close to the implant, the extracellular space contained a second type of fluid. The latter was thought to be of hematogenous origin. Fluid of this type was the essential element of the pool of fluid produced in response to the intracerebral implantation of silver nitrate.

This research was presented at the Annual Meeting of the Federation of Western Societies of Neurological Sciences held on Sunday, 7 March 1965 at the Mirador Hotel, Palm Springs, California.

A further study is to be presented at the Workshop Symposium on Brain Edema, Vienna, 11-13 September 1965 following the 8th International Congress of Neurology.

Part B included: No

Serial No. NDB (CF) - 64 E 1250
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Reaction of the Nervous System to Cryptococcal Infection. An Experimental Study with Light and Electron Microscopy.

Principal Investigators: S. Levine
New York Medical College Center for Chronic Disease
A. Hirano

Other Investigator: H. M. Zimmerman
Montefiore Hospital

Cooperating Units: New York Medical College Center for Chronic Disease, New York, New York
Montefiore Hospital, New York, New York

Man Years
Total: 1/2
Professional: 3/8
Other: 1/8

Project Description:

The cells of cryptococcus neoformans, whether living or dead, elicited only a weak and delayed inflammatory reaction in the brains of experimental animals and some human patients. This is due, at least partly, to an inherent quality of brain tissue, since the cryptococcal organisms often caused vigorous inflammation in other organs. The cryptococcal capsule and cell wall were extremely resistant to digestion, even when engulfed by phagocytes in the brain. The purified capsular polysaccharide implanted into the brain enjoyed the same relative freedom from inflammatory reaction and from digestive degradation. It is likely that both the peculiar qualities of the capsular polysaccharide and the inherent characteristics of brain as a tissue result in an inadequate inflammatory response to the cryptococci. This may be partly responsible for the predilection of cryptococcosis for the brain, and for the chronic progressive character of the infection. Other factors inherent in the organism and in the biochemistry of the nervous system are undoubtedly important, as well.

This research was presented at the 44th Annual Meeting of the Association for Research in Nervous and Mental Diseases, New York, New York, December 5, 1964.

Part B included: No

Serial No. NDB (CF) - 64 E 1251
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Amyotrophic Lateral Sclerosis, Histopathological Considerations

Principal Investigator: A. Hirano

Other Investigator: L. T. Kurland

Cooperating Unit: Montefiore Hospital, New York, New York

Man Years
Total: 1/4
Professional: 1/8
Other: 1/8

Project Description:

This study covers the following five points:

1. The histopathologic findings in classic, sporadic cases of ALS based on a review of material at Montefiore Hospital.

2. Familial ALS in which the classic findings are frequently noted, but in some cases of which posterior-column demyelination and other atypical changes may occur.

3. Histopathologic features observed in Guam ALS cases.

4. Neuropathological features of P-D Complex on Guam, with special reference to Lewy's bodies in substantia nigra and electron microscopic study of fibrillary structures in the pyramidal cell layer of the Sommer's sector.

5. On the eosinophilic inclusion bodies observed and described in hereditary ALS from the U.S.S.R. by Bunina. Studies of spinal cord sections from the experimental monkeys used in studies by Zilber and colleagues in Moscow.

Part B included: Yes

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Proceedings of Workshop Symposium on Latent, Slow and Temperate Virus Infections, NINDB, NIH, Bethesda, Maryland, December 7-9 1964. U.S. Government Printing Office (in press).

Honors and Awards relating to this project: None

Serial No. NDB (CF) - 65 E 1252
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Reaction of Ependyma to Cryptococcal Polysaccharide Implantation.

Principal Investigator: A. Hirano

Other Investigators: H. M. Zimmerman
Montefiore Hospital
S. Levine
New York Medical College Center for Chronic Disease

Cooperating Units: Montefiore Hospital , New York, New York
New York Medical College Center for Chronic Disease , New York, New York

Man Years

Total:	1/2
Professional:	3/8
Other:	1/8

Project Description:

Following intracerebral implantation of cryptococcal polysaccharide, large amounts of polysaccharide-rich fluid spread into the distended extracellular spaces of the subependymal region. Alterations of ependyma and subependymal structures were studied with light and electron microscopy after the whole brain was fixed in situ by perfusion. Considerable amounts of fluid were engulfed by subependymal phagocytic cells, while smaller amounts entered the cytoplasm of ependymal cells usually as small cystic collections. The adjacent ependymal cells generally maintained close contact, without obvious separation. Phagocytes with engulfed fluid, nevertheless, were capable of separating adjacent ependymal cells and were able to traverse the ependymal barrier into the lumen of the ventricle.

This report will be presented at the Annual Meeting of the American Association of Neuropathologists on June 13, 1965 in Atlantic City.

Part B included: No

Serial No. NDB (CF) - 64 E 1253
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Influence of small systematic errors on the results of tonometric screening.

Principal Investigator: J. T. Schwartz

Man Years

Total:	1/16
Professional:	1/32
Other:	1/32

Project Description:

Although glaucoma surveys have been performed among many populations, relatively few reports are truly comparable. In this study a graphic method was used to demonstrate the important influence of small differences in tonometric techniques on the outcome of mass tonometry surveys.

The frequency distribution of ocular tensions among a population of 414 retired persons was used to demonstrate that a systematic error of only 2 mm. of mercury would alter the proportion of positive screenees at 25 mm. of mercury by about 60%. The amplitude of potential systematic error resulting from a number of differences in tonometric techniques was estimated from available literature. The influence of these error values on tonometric survey results was then determined by graphic methods.

It was found that differences in examination procedure which might ordinarily hold minimal significance in clinical practice can exert considerable influence on the outcome of population surveys. This study concluded that a single, specific, and carefully defined tonometry technique must find broad acceptance in order to acquire comparable geographic data on the frequency of ocular hypertension.

Part B included: Yes

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Schwartz, J. Theodore: Influence of small systematic errors on the results of tonometric screening. American Journal of Ophthalmology (in press).

Honors and Awards relating to this project: None

Serial No. NDB (CF) - 64 E 1254
1. Collarobative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Comparison of glaucoma response following steroid provocation among monozygous and dizygous twins.

Principal Investigator: J. T. Schwartz

Man Years:

Total:	1/8
Professional:	1/16
Other:	1/16

Project Description:

Comparison of concordance among monozygous and dizygous twinships with respect to a specific physical characteristic provides an evaluation of the relative role of genetic or environmental influence underlying the development of the characteristic.

A registry of local twins who will be available for ophthalmic examinations on an outpatient basis is being developed by the ophthalmology field and collaborative research activity. During the past year over 200 twin pairs of all ages have been located as potential outpatient examinees.

Proposed course of project: An ophthalmic screening examination and zygosity determination will be provided for the twin population. Selected twin pairs will be scheduled for definitive examinations. The first investigation will undertake a comparison of the concordance among monozygous and dizygous twinships with regard to clinical parameters of glaucoma. A comparison of the response to topical steroid provocation will be included in the glaucoma evaluation.

Part B included: No

Serial No. NDB (CF) - 64 E 1255
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Tonometry survey in an Italian-American population.

Principal Investigator: J. T. Schwartz

Other Investigator: G. G. Dell'Osso
Biometrics Branch, NINDB

Cooperating Unit: Biometrics Branch, NINDB

Man Years

Total:	3/16
Professional:	1/8
Other:	1/16

Project Description:

A community-wide tonometry survey was performed among adult Italian-Americans living in Nesquehoning, Pennsylvania. Americans of Italian descent living in a nearby Pennsylvania county are reported to experience a low mortality due to coronary artery disease. Nesquehoning was selected for pilot investigation largely because of our ultimate interest in investigating the possible association between chronic simple glaucoma and chronic vascular disease (see Project No. NDB (CF) - 63 E 1122).

This survey was undertaken with the following specific objectives: (1) to establish the frequency distribution of ocular tension in a defined Italian-American population, (2) to determine postural influence (sitting vs. supine) on applanation tonometry measurements in a defined "total population," and (3) to compare the results of applanation and Schiotz tonometry in a defined "total population."

The investigation was undertaken in the form of a mass survey program. Community participation was organized through the cooperation of a team of local volunteers. The target population was 450 persons age 25 and over, of whom 84 percent were examined. Positive screenees were subsequently referred to attending physicians for definitive care.

Data are being analyzed with respect to the three objectives.

Part B included: No

Serial No. NDB (CF) - 64 E 1256
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Tonometry survey in an American Indian population.

Principal Investigator: J. T. Schwartz

Other Investigators: G. R. Williams
G. G. Dell'Osso
Biometrics Branch, NINDB
D. Powers
Division of Indian Health
B. Becker
Washington University School of Medicine
N. Ballin
Washington University School of Medicine
M. Newman
Washington University School of Medicine

Cooperating Units: Biometrics Branch, NINDB
Division of Indian Health, Phoenix, Arizona
Washington University School of Medicine,
St. Louis, Missouri

Man Years
Total: 1/4
Professional: 3/8
Other: 1/8

Project Description:

American Indians have been reported to have a low frequency of chronic simple glaucoma. This study was a reservation-wide tonometry survey at the Colorado River Indian Reservation in Arizona, whose Indian population is of mixed tribal origin. Ocular tensions were measured on each subject by three different techniques: Goldmann applanation in the sitting position, Goldmann applanation in the supine position, and Schiotz indentation in the supine position.

The primary objectives of this survey were as follows: (1) to obtain pilot information on the frequency distribution of ocular tensions among a defined population of American

Indians, (2) to provide a second evaluation of the influence of postural differences (sitting and supine) and instrument differences (Goldmann applanation and Schiotz indentation) on tonometric measurements, in order to provide a cross comparison with similar data previously obtained in a separate population (see Project No. NDB (CF) - 64 E 1255), (3) to correlate the results of tonometric screening by each of three techniques with the results of a definitive clinical evaluation for glaucoma, and (4) to appraise the physical practicability of employing Goldmann applanation tonometry, sitting or supine, in population surveys performed under rigorous field conditions requiring a moderate degree of mobility.

The field investigation was undertaken as a two-and-a-half week mass screening program. During this period the survey teams operated from five examination sites on the reservation. Community participation was organized by a field team from the Division of Indian Health. Diagnostic evaluation, including ophthalmoscopy, tonography and gonioscopy, was performed by a field team from Washington University. Tonometry was performed on 439 patients. Approximately 87 percent of the target population of adults age 30 and over were examined.

The data are being analyzed for Objectives (1) and (2), and the correlations in Objective (3) will be made when data from the glaucoma work-up becomes available.

The survey provided an opportunity for thorough appraisal of the practicability of performing applanation tonometry in the field using the applanation tonometer with slit lamp (sitting) or operating microscope (supine). Use of either method was satisfactory even under fairly rugged field conditions [Objective (4)].

Part B included: No

Serial No. NDB (CF) - 64 E 1257
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: U. S. mortality experience with malignant neoplasms of the eye.

Principal Investigator: G. R. Williams

Other Investigators: J. T. Schwartz
I. D. Goldberg
Biometrics Branch, NINDB

Cooperating Unit: Biometrics Branch, NINDB

Man Years

Total:	1/4
Professional:	1/8
Other:	1/8

Project Description:

Under the present system of classifying causes of death, all fatalities due to malignant neoplasms of the eye are assigned without subclassification to Rubric 192. Retrospective characterization of eye tumor mortality by histologic type requires, therefore, an examination of individual death certificates.

In the United States during the period 1959 to 1961, there were 1,108 certificates in which the primary cause of death was assigned to Rubric 192. A full copy of the microfilm image of these death records will be provided by the Division of Vital Statistics, National Center for Health Statistics. The data from these records will be recorded in a standard format and will subsequently be analyzed in collaboration with the Biometrics Branch. Certain epidemiologic features of eye tumor mortality in the United States such as age, race, sex and state of residence will be obtained from this analysis.

It is anticipated that additional information will be solicited on certain cases, and where necessary an attempt

will be made to recover surgical specimens or autopsy materials. Dr. Lorenz Zimmerman, Chief, Ophthalmic Pathology Branch, Armed Forces Institute of Pathology has agreed to examine the pathologic material which we recover. Attending physicians, referring physicians, hospitals, clinics, tumor registries, and other sources of professional information will be contacted in order to obtain data on cases which are of special importance.

Part B included: No

ANNUAL REPORT
For Period July 1, 1964 through June 30, 1965
Perinatal Research Branch
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

I. GENERAL STATEMENT

Summary of Professional Achievements

The Perinatal Research Branch during the past year continued its major activity and responsibilities as the coordinating unit of the Collaborative Project on Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood. With data collection proceeding on schedule the Project has enrolled at this stage approximately 55,000 pregnancies and will terminate intake to the present program with a projected goal of 60,000 pregnancies by January 1, 1966. While intake to this Project is coming to a close during the next fiscal year, program development continues and batteries of examinations to define neurological, psychological, language, speech and sensory deficits in children at seven to eight years of age have just been developed. Plans are formulated for the continued assessment of this population of children through age 12 for the purpose of documenting precisely specific learning disabilities so commonly detected in the school situation for the purpose of assessing whether such specific learning disorders are indeed the result of prenatal or immediate postnatal events.

With a considerable improvement in the availability of program and computer capability the analysis of the data from this Project has progressed considerably during the past year.* These analyses have dealt with many characteristics of pregnancy - obstetrical and medical complications - in relation to perinatal mortality and neurological morbidity which can be defined in certain children during the first year of life. The results of these investigations underscore once more the complexity of the problem since it can be shown now that a large number of factors of pregnancy are associated with an increase in neurological deficits in surviving children with considerable interaction of many of these variables.

The strong and well-known association of prematurity with neurological deficits can be substantiated with the data from the Collaborative Project which reveal a threefold increase in neurological deficits in prematures when compared to term children. The specific motor deficits of spastic diplegia and quadriplegia appear to be even more strongly associated with prematurity. While the analysis of the data related to the problem of prematurity have as yet failed to demonstrate that the

* See page 7.

increase in neurological deficits in prematures is largely the result of the prematurity rather than related to the cause of prematurity, some support can be given to the hypothesis that neurological deficits in a high proportion of prematures arise out of an interaction of certain prenatal factors with certain problems characteristic of prematures.

Factors of pregnancy such as premature separation, placenta previa and cord prolapse which potentially may produce anoxia in the fetus quite surprisingly are found to result in only a mild increase in neurological morbidity among surviving children in contrast with the approximately ten-fold increase in perinatal mortality associated with these conditions. The neurological findings in these children if present are rather non-specific with conspicuous absence of clear-cut motor deficits. This may suggest that most of the survivors recover from the insult and are normal. It is more likely however that these conditions, while not leading to cerebral palsy, may in fact be associated with an increase in learning and/or behavioral deficits,possibly some decrease in IQ, effects that cannot be identified clearly at one year of age.

Among fullterm children, it is noted that complications of labor appear to increase neurological deficits among children. Examples of these complications are maternal and fetal dystocia, prolonged labor, cephalo-pelvic disproportion and the like. The study of medical complications of pregnancy which may affect the fetus suggests that glomerulo-nephritis, chronic hypertensive disease, diabetes, hyperthyroidism, bronchial asthma(symptomatic)in pregnancy, organic heart disease with decrease in functional capacity result in an increase in perinatal mortality and neurological deficit. It is also to be noted that neurological deficits were found to be considerably increased among women who appear to be mentally retarded, among women who had epileptic seizures during pregnancy, in contrast to those whose seizures were controlled with medication during pregnancy.

A considerable effort has been made to identify more precisely the child who is likely to be abnormal by utilizing various characteristics observed during the newborn period. Of measures that we have found useful, particularly noteworthy are five-minute Apgar scores. In addition abnormalities of the grasp reflex, and of sucking, appear to predict neurological deficits in the small premature. Numerous other signs and symptoms of a neurological nature identified in newborn children are similarly evaluated.

Investigations of infectious diseases in pregnancy and their effect on the fetus indicated that congenital toxoplasmosis may occur in about one out of two thousand study children. A unique observation is the demonstration of congenital toxoplasmosis in a child who appears to be, on the basis of all of the available evidence, collected during the first year of life, perfectly normal. The rubella epidemic which occurred on the East Coast and the Southwest of this country

particularly during the early part of last year, was monitored in part by the Collaborative Project. Through these efforts the Study was able to document and confirm reports of attack rates in the Study population, congenital malformations related to clinical rubella, the persistence of the virus in the fetus and newborn affected by congenital rubella, the unreliability of the reporting of exposure and/or clinical rubella when evaluated in terms of sero-conversion and the frequency and effect of inapparent rubella. Studies of the current rubella epidemic in related populations of Hawaii are in progress.

Based on leads developed by this program a series of studies of specific disorders of pregnancy are planned which should be ready for pretest in Fiscal 1966 Such studies will utilize in part a collaborative framework as necessary or will be conducted on a cooperative basis if appropriate.

Publications

Berendes, Heinz W.: Obstetrical Complications and Mental Deficiency. International Copenhagen Congress on the Scientific Study of Mental Retardation, Denmark 7-14 Aug. 1964.

Benson, Ralph C.; Shubeck, Frank; Clark, William M.; Berendes, Heinz; Weiss, William; and Deutschberger, Jerome: Fetal Compromise During Elective Cesarean Section. A Report from the Collaborative Project. American Journal of Obstetrics and Gynecology, Vol. 91, No. 5, March 1, 1965.

Berendes, Heinz W.; Weiss, William; Deutschberger, Jerome; Jackson, Esther: Factors Associated with Breech Delivery. American Journal of Public Health (in press).

Berendes, Heinz W.: The Evaluation of Therapeutic Procedures in the Practice of Obstetrics. Proceedings of IVth World Congress of the International Federation of Gynecology and Obstetrics, Buenos Aires, Argentina, September, 1964 (in press).

Berendes, Heinz W.; Weiss, William; and Deutschberger, Jerome: Evaluation of the Problem of Breech Delivery. Proceedings of the Symposium on Effects of Labor on the Fetus and Newborn, Montevideo, Uruguay, October, 1964 (in press).

Administrative Achievements

As of April 30, 1965, about 55,000 women have been registered in the Study, about 47,000 have been delivered, about 31,000 births have been studied at one year of age, about 8,000 at three years of age and about 7,500 at 4 years of age. By the middle of FY 1966, the obstetrical intake in the current phase of the Study shall have stopped and an expected 60,000 women would have registered.

The available data from 2,200,000 Study forms thus far received, have been transformed into three million punch cards, 91 different card files, and converted to computer tape files. Out of a total of 65 different Study forms already designed, there is no backlog in editing or coding except for three forms (OB-45, PATH-1 and 2). In addition, routine feedback to all institutions, on pooled data, has taken the form of frequency and cross tabulations, etc. (Appendix C)

There is sufficient evidence that the quality and quantity of data as collected, in accordance with established protocol on all forms and items thus far adopted in the Study, are very adequate for a multiplicity of analyses geared to meaningful testing of relevant hypotheses or to justified fishing for new ones.

The Project completed, during this year, the development of four retrieval systems to handle large masses of information or data. The centralized facilities of the Project in the Infectious Diseases Section, Pathology Section, Systems Design and Procedure Section and the Medical Literature Services Unit are the foci where these retrieval systems have been developed. The Section on Infectious Diseases receives, stores, classifies, retrieves and analyzes a multitude of sera, of tissue specimens and of antigens in a systemized pattern under control; the Section on Pathology receives, stores, classifies, retrieves and analyzes, via a computer-based system intended to tabulate as well as diagnose, hundreds of pathology specimens; the Section on Systems Design and Procedures receives masses of research forms and data, classifies them, edits and codes them, card punches and creates computer files on them, stores them and selectively retrieves them at short notice; the Medical Literature Services Unit has developed a system for mechanical retrieval of reprints needed in review of literature based on topics selected and classified for preparation of papers in the perinatal area. In addition, the development of a system to retrieve administrative and professional decisions is being explored.

The inadequacy of computer facilities that was mentioned in last year's report is no more. PRB, through NINDB and NIH, has been able to avail itself of the NIH computer facilities, as well as of non-government computer facilities by contract, to meet on a continuous basis, the increasing load of requests for data analysis. In addition, a substantial effort is made at PRB for hand abstraction of data on selected proposals based on case studies, which effort is centralized in the Section on Statistical Analysis.

A document on the history of the Project since the seed of its idea was planted in 1952, is being prepared and which hopefully will be finished during the coming fiscal year.

The Branch, through a number of its personnel, has participated in a number of panels and presentations in the U.S.A. and outside. (Appendix D)

The Publications Review Board continues to function as efficiently as ever. It has reviewed 18 papers during this fiscal year (Appendix A).

During this fiscal year 52 or more study proposals (Appendix B) have been submitted to cover the following areas: labor and delivery, infections in pregnancy, specific pregnancy complications, selected predictors of neurological deficit in pregnancy outcome, correlation of prior pregnancy factors with subsequent pregnancy course and outcome, medical correlates of obstetrical factors, and selected antenatal factors related to fetal loss and congenital malformations, etc.

During this year, new instruments have been finally adopted: the Maternal I.Q., the 7-8 Year Battery of Tests including pediatric-neurology, psychology, speech, language and hearing, coarse visual and auditory screening tests and FHH-9. In addition, VIR-2, 3 and 4, have been adopted.

PRB continues, as ever, to be the focal point for evaluation of data collection, the activities of the Executive Board, the Data Analysis Board, Publications Review Board, the Project Directors' Committee, the various professional coordinators' committees, and any other ad-hoc task forces established.

During this year a decision was made to extend the follow-up of the current phase of the Study to age 12. This extension would involve the development of new instruments in pediatric-neurology, psychology, speech, language and hearing, socio-economics, vision and hearing, and especially the creation of a new instrument to evaluate the educational performance of the child in a further attempt to define central nervous system damage, not only on the basis of perinatal information thus far collected, but also on the basis of information derived from an adequate assessment of the school performance of the child. Experts in the field of psychology, speech, language and hearing, education and pediatric-neurology have agreed that the educational experience of the child, in school, may be indispensably helpful in diagnosing areas of brain damage which otherwise cannot be deciphered with the other instruments alone.

Of special importance to the Study is its activity and achievement in dissemination of information to the lay public, as well as to Congress. After all, it is this citizenry that has asked for the Project and continues to support it. In a true sense, the Project data are in the public domain.

It would have been a surprise if the masses of data available in the Project did not challenge the statisticians on the staff. Whereas the majority of study proposals can be handled by routine statistical methods, there are areas which call for the creation of new techniques which when fully developed, would constitute in themselves a contribution to the field of statistical theory and methodology.

It is quite relevant to state that both the Perinatal Research Committee and the senior Principal Investigators in the Project have continued to express their faith and support. Nevertheless, it is fair to mention that the Perinatal Research Committee has given more explicit support and guidance than the Principal Investigators as such.

An EEG primer on the normal neonate has been developed during this year, thanks to the efforts of PRC and Dr. Kellaway. The abnormal EEG primer is forthcoming.

Experience gained thus far in the Project has amply demonstrated that whereas collaboration between institutions and disciplines at various levels of operation has taken place to some extent during the design and data collection phases of the Study, this collaboration is conspicuously coming to the foreground as an absolute necessity, despite its problems and pains, in the phases of data analysis and publication. Therefore, when the final administrative story of this Project shall have been written, it will have a specific contribution to offer as to the specific mechanisms by which various tasks have been delegated and various other coordinating and integrating decisions have been made.

Problems

Besides the problems inherent in Collaborative Research which have been referred to in previous annual reports, e.g., effective decision-making amidst a number of committees, task forces, institutions and disciplines, this Project at PRB faces the problem of shortage of personnel and of funds to meet its growing needs in the areas of: decentralized computer facilities, increasing concern with the disciplines of education, sociology, virology, cytogenetics and speech, language and hearing. In addition, more space is needed for cytogenetic studies, computer facilities and experimental animal work.

With regard to the Future of the Study, there is evidence that the Principal Investigators and Project Directors from all collaborating institutions have demonstrated ample concern about the termination of OB intake in the current phase of our Project as of January 1, 1966. A special task force has been established to stimulate, produce and deliberate on proposals for future studies. It is anticipated that by the end of FY 66 enough leads shall have emerged from the present data to help in planning for the future. Furthermore, the current phase of the Study continues with an extended follow-up of children to age 12, incorporating the development of new instruments to assess the school performance, as an adjuvant to a more specific definition of neurological deficit between ages 5 and 12.

The Second Scientific Meeting, late in January 1966, will present a number of finished papers on such topics as medical problems of pregnancy, predictors of neurological damage, high risk pregnancies and

correlation of evidence on neurological status derived from a variety of instruments at stated intervals in the natural history of growth and development, etc. Whereas the follow-up to age 7 will reveal specific neurological deficits, discernible by that age, through an interdisciplinary diagnostic summary of all tests performed, the subsequent follow-up to age 12 will, in addition to sharpening the 7-year endpoint, discover and define other parameters of neurological deficit, largely through the help of the assessment of school performance, and will also hopefully reveal the impact, if any, of the socio-economic environment on the growing child as reflected in measurements taken prenatally, at repeated intervals after birth, especially at age 7 and at age 12. Furthermore, the extended follow-up might be helpful in discovering parameters to assess the process of learning per se, in the growing school child.

In this total scope of the Project, based on the current OB intake and follow-up to age 12, the genetic, biological, psychological, educational and socio-economic parameters of assessment of growth will have been developed and brought to focus on the initial objective of the Study, namely evaluation of impact on neurological status of the child, of prenatal, perinatal and postnatal factors, as defined.

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* Refers to page 1.

With reference to the individual Project reports, reflecting research activity emanating directly from the Office of the Chief, PRB during the current fiscal year, it is appropriate to mention that (a) three individual Project reports of last year, Serial No. NDB (CF)-63 PR/OC 1141, 1142 and 1143, have actually been written as papers, (b) individual Project reports, Serial No. NDB (CF)-63 PR/OC 1145 and 1147, are still in progress by Miss H.R. Pietrowski who is pursuing her nursing education at Columbia University, (c) the majority of study proposals listed under Appendix B warrant to be described as individual Project reports under the Office of the Chief, but for the sake of brevity their full description as individual Project reports will appear in next year's report, when they shall have matured either into full manuscripts or shall have been processed more than at present, in that general direction.

I. Summary of Scientific or Professional Accomplishments

A. Scientific Activities

Gynecologic History, OB-4

The date of the last menstrual period (LMP) prior to conception is a critical piece of information for all studies using gestational age as a variable. Because this data depends primarily on the information reported by the mother, it carries all the vagaries and inconsistencies of historical information. Frequency distributions of LMP by race, age of mother, and time of registration were studied to define criteria and limits for an acceptable LMP for Study cases. Obstetric and Pediatric Coordinators joined the staff of the Section for one full work week to review nearly 5,000 obstetric records in which discrepancies between gestational age and birthweight were noted.

Fertility, OB-4

Coding instructions were written for the history on fertility, sterility, and the use of contraceptives in the Study population. Preliminary tabulations of the data from these areas let us predict that the study will produce relevant information on the association of fertility and sterility factors with pregnancy outcome.

Special Studies (See Individual Project Reports)

1. Methods and problems in the epidemiologic study of perinatal data (Yale Report).

2. Criteria for the acceptance or rejection of gestational age data by birthweight.

3. Review of the literature on toxic effects of lead and lead compounds on the mother and child during the perinatal phase.

4. Possible relationship of subnormal semen to the outcome of pregnancy.

5. Bibliography entitled "Fifty Years of Research on Mammalian Reproduction," Public Health Service Publication No. 1281, Public Health Bibliography Series No. 57.

Pilot Studies

1. Hyperemesis Gravidarum

Based on a printout from the Obstetric Summary (OB-60), 443 cases of hyperemesis gravidarum, I.V. therapy, and acetonuria were reviewed and analyzed. It was found that the reporting of this condition varies greatly between hospitals and with the time of registration.

2. Rubella During Pregnancy

Three members of the nursing staff participated in a record review of cases reporting exposure to rubella during the present Study pregnancy in the following collaborating institutions: Boston Lying-in Hospital, Charity Hospital, in New Orleans, Louisiana, and Providence Lying-In Hospital.

B. Administrative Activities

Editing and coding of obstetric forms including: OB-2, 3, 4, 5, 6, 7, 8, 9, 10, and 42; editing OB-42, 43, 51, and 60; and abstracting OB-32, 33, and labor and delivery drugs.

Training sessions for medical editors from collaborating institutions and for the staff of the Section.

Professional staff seminars.

Project site visits to collaborating institutions.

Review of obstetric records for evaluation of the performance of the collaborating institutions.

Preparation of data for Obstetric Coordinators' and Project Directors' meetings.

Assistance to collaborators and perinatal research staff in the design and preparation of research papers.

Writing of obstetric papers and reviews of the literature on specified perinatal subjects.

Consultation and cooperation with other agencies and individuals on specific problems in the perinatal research area: National Institute of Child Health and Human Development, Food and Drug Administration, Margaret Sanger Research Bureau for Planned Parenthood, Population Reference Bureau, German Study Group of Teratology, Polish Perinatal Research Group "Mother and Child," Perinatal Study in Hawaii.

Instruction and guidance of medical summer students in the design and execution of an epidemiologic study on perinatal data.

The Section was host to the following guest speakers:

Dr. Carl G. Hartman, Research Director, Margaret Sanger Research

Bureau, who reported on the opossum as a research tool in reproductive physiology.

Dr. Matthew M. Block, Professor of Medicine, University of Colorado Medical School, who lectured on new findings in the embryology of the thymus gland and its relationship to feto-maternal interactions.

Dr. Seymour Katsh, Acting Head, Department of Pharmacology, University of Colorado Medical School, who lectured on immunologic reactions involved in the processes of fertilization and implantation.

Three scientific films were shown as part of the training program for the staff:

1. Normal Delivery (American College of Obstetrics and Gynecology)
2. Vacuum Extractor (Malmstrom)
3. Implantation Mechanism in the Guinea Pig (Blandau)

Approximately the following number of forms were edited and/or abstracted in the Section as of April 1965:

	Completed	Backlog
OB-60, Obstetric Summary	10,100	2,000
OB-55, Delivery Report	5,700	3,500
OB-32 & 33, Labor and Delivery and Drug Abstraction	15,800	5,400
Prenatal Drug Abstraction	10,100	800

The conversion of OB-34 (labor and delivery report) into the revised OB-55 was discontinued after it had been demonstrated and discussed with the Obstetric Coordinators and Project Directors (see Information Memo No. 185 of September 14, 1964) that there are major areas in the contents of these two forms which contain incongruous information that cannot be converted without risk of distortion of the original data. Basic information on labor and delivery is adequately available from both forms, but certain special data can be drawn from OB-55 only.

II. Problems

The internal routine operation of the Section (coding, editing, and abstracting of obstetric records) is functioning smoothly. While the Section has a good and regular correspondence with all the Obstetric Coordinators in the collaborating institutions concerning data collection, the interest of the obstetricians in the analysis of the data needs to be stimulated and developed.

III. Proposed Future Objectives

To explore:

A. the combination of geographic and racial factors on the course and the outcome of pregnancy (abortion, fetal and neonatal deaths,

major complications of pregnancy, labor, and delivery),

B. the degree of variability in the reported length of the menstrual cycle and the duration of the Study pregnancy,

C. the use of different types of contraceptives in relationship to the outcome of the Study pregnancy,

D. the gynecologic age versus the chronologic age of the mother in relationship to the course and outcome of pregnancy (incidence of pregnancy complications, birthweight, duration of pregnancy, labor and delivery complications, incidence of abortions, fetal and neonatal deaths).

IV. Remarks

Personnel

The needs of the Section are very closely tied in with personnel. There are two vacancies of long standing for medical officers. Nine interested and qualified candidates have been interviewed during the past eight months, all declined because the Section could not offer any clinical or laboratory facilities. It seems legitimate that the young research obstetrician does not want to lose his clinical experience and his operative or laboratory skills by becoming tied completely to a desk job on epidemiologic studies. I feel very strongly that for attracting qualified researchers--and for the benefit of the Study--we should plan to acquire laboratory facilities for work in experimental reproductive physiology. There are several important areas that could be attacked, even with limited means: the aging factor in reproduction in small animals, specified nutritional factors (protein deficiency, experimental diabetes), the male factor in duration of pregnancy and pregnancy wastage.

Organization

No change so long as OB intake continues.

Space

Except for laboratory facilities, no additional requests at the present.

B. SECTION ON PEDIATRIC NEUROLOGY
Report for Period July 1, 1964 through June 30, 1965

I. SUMMARY OF SCIENTIFIC OR PROFESSIONAL ACCOMPLISHMENTS:

A. The Section staff has been working intensively with a task force of five pediatric neurologists on the development of the Seven-Year Pediatric-Neurologic Examination. In addition, the Pediatric Section has been responsible for the coordination of the seven-year battery, including the psychological examination as well as a speech, language and hearing examination to be done at eight years. This coordination has included a review of the primary areas to be examined by each of the disciplines in order to insure adequate coverage, and to minimize overlap. The work with the Task Force on the Pediatric-Neurologic Examination has progressed rapidly. The examination form and manual have been pretested by Dr. Richmond Paine at the Children's Hospital in Washington, D. C., and by Dr. Charles Kennedy, Pediatric Neurologist at The Children's Hospital in Philadelphia, Pennsylvania. The examination is a general pediatric and neurologic examination which includes tests for near and far vision, color visual screening, an estimate of mental function, and an estimate of intellectual function. The examination form and manual must be presented to the Project Directors of the Study for their approval, and must be in final form for adoption as Study protocol as of January 1, 1966 when the first children of the Core Study program will reach age seven.

B. The completion of the backlog intake of PED-8 (Newborn Diagnostic Summary) has been accomplished during this fiscal year. This has made available a large number of diagnoses emanating from the newborn period for data analysis. The PED-8 is a semi-coded summary in which are recorded significant diagnoses or events which occurred during the interval from birth to discharge from the nursery hospital. Having the PED-8 available has greatly enhanced the ability of the Branch to define outcomes and various types of protocols. In addition, it will greatly facilitate the current drug study in that pregnancy outcomes are easily and rapidly identified from the PED-8.

C. There has been marked progress in the backlog intake of the PED-12 (Summary of the First Year of Life). This backlog should be substantially completed by July 1, 1965, the deadline set by the Project Directors. Approximately 30,000 PED-12 examinations should then be available for data analysis. With the accumulation of PED-12's within the Perinatal Research Branch, the Section participated in the construction of a coding structure for the PED-12, and this is covered in detail in Item F. Coding has begun within the Section on Statistical Procedures and Analysis and this Section continues to work with that Section in solving problems and correcting information as the coding of PED-12 proceeds.

D. A bibliography, entitled "Pediatric Neurology as it Relates to Cerebral Palsy and Mental Retardation", has been prepared for distribution within the Project and to other interested persons. Within this bibliography, there are twelve general divisions and some 512 references. The division of the references is as follows: Early Signs; Neonatal Asphyxia; Neonatal Convulsions; Kernicterus; Prematurity, Dysmaturity and Growth Retardation; Maternal and Obstetrical Factors as Related to Outcome; Behavioral Studies; Minimal Brain Damage in Children; Sensory and Autonomic Functions and Conditioning; Special Tests; Physiology, Anatomy and Pathology; and General; plus an Addendum. It is expected that this bibliography will facilitate the planning of scientific studies and the production of scientific documents. It is intended that this bibliography will be continued and will be available as a service to our collaborators.

E. During the fiscal year 1965, there has been a continued input of pediatric protocols; and as of April 2, 1965, there were some 649,365 pediatric forms in the Perinatal Research Branch files. The input of pediatric protocols will continue at its present rate until the obstetrical intake is discontinued as of January 1, 1966. As the number of births gradually diminishes in 1966, the last set of nursery protocols will be completed. In addition, in fiscal year 1966 we anticipate that most of the four-month examinations (PED-10) will have been performed. The completion of both the nursery set and four-month examination will decrease the rate of influx of these forms to our files, but the overall rate of input should continue since Study children will begin their seven-year examination and the diagnostic summary covering diagnoses and events from one year to seven years will be received by the Perinatal Research Branch.

F. The Section on Pediatric Neurology participated in the preparation of the PED-12 (Summary of the First Year of Life) coding structure. The PED-12 serves as a summary of diagnoses and events occurring after the date of discharge from the nursery through the one-year examination, or 60 weeks of age, whichever comes first. The PED-12 is also precoded, in that the physician completing the PED-12 at the collaborating institution checks code numbers corresponding to specific diagnoses, procedures or events. This form is then received by the Perinatal Research Branch and the information can be rapidly converted to punch cards which are readily available for data analysis. It allows the physician completing the diagnostic summary to determine just what information is being put on the tape files and eliminates any clerical judgment as to whether or not a diagnosis or event should or should not be coded. However, this coding is verified when these forms are reviewed at the Perinatal Research Branch, where they are checked by statistical clerks; then, if necessary, referred to the medical staff. The Perinatal Research Branch file is reviewed and a check is made to see that the material within it has been accurately coded on to the PED-12. This increases the accuracy of the reporting on the PED-12. When discrepancies are encountered, erratas are sent to the Collaborating Institutions for the necessary revisions.

G. The Section on Pediatric Neurology has continued to participate in data analysis. This participating ranges from advising collaborators regarding Study plans to the actual designing, implementing, and completing of the study by the Section. The topics that are being studied are reported in detail within the individual project reports in the appendix. There are some thirteen of these currently undergoing study. The studies in which members of the Section are participating include:

1. Prematurity. (Serial No. NDB-(CF)-63 PR/PN 1161)

2. Apgar Score - An Index of Infant Morbidity. (Serial No. NDB-(CF)-63 PR/PN 1155)

3. Neonatal Hyperbilirubinemia and Subsequent Neurological Sequelae. (Serial No. NDB-(CF)-63 PR/PN 1156)

4. Apgar Scores. (Serial No. NDB-(CF)-63 PR/PN 1162)

5. An Investigation Into the Relationship Between Congenital Heart and Great Vessel Anomalies and Selected Perinatal Factors as Recorded in the Collaborative Perinatal Research Project. (Serial No. NDB-(CF)-63 PR/PN 1163)

6. Early Signs of Neurological Abnormality in Newborn Infants. (Serial No. NDB-(CF)-63 PR/PN 1164)

7. Clefts of Lip and Palate as Indicators of Perinatal Morbidity. (Serial No. NDB-(CF)-63 PR/PN 1165)

8. Toxoplasmosis: Serological and Clinical Studies.

9. Maternal Rubella and Pregnancy Outcome.

10. Hyaline Membrane Disease.

11. A Study of Socioeconomic, Medical and Genetic Factors in Major Congenital Malformations. (Serial No. NDB-(CF)-63 PR/EG 1173)

12. Retrospective Georgetown Study. (Serial No. NDB-(CF)-63 PR/OC 1144)

13. Aplasia of One Umbilical Artery. (Serial No. NDB-(CF)-63 PR/PN 1159)

In addition, the Section has participated in a number of other studies in which they have performed case study reviews. These include studies on placenta previa, abruptio placenta, bleeding in the first trimester, incompetent cervix, anemia, blood types and outcome, and outcome after drug injection during pregnancy.

H. The Section has participated in the discussion concerning the extention of Study follow-up to age twelve. This follow-up is primarily justified on the basis of gaining information regarding the school performance of Study children. This type of evaluation at twelve years would obtain information from interviews with teachers, inspection of school records, and actual tests of educational achievement. In addition, a psychological examination at twelve years and a pediatric examination at twelve years should be considered. It is anticipated that socio-economic data would be collected during this period of time. There also have been several proposals for a more detailed eye examination at about eight years to be followed by another ophthalmoscopic examination at twelve years. The two eye examinations would document the changes in refractive error over this interval of time and possibly relate some of these refractive errors to earlier pediatric or socio-economic data. It is anticipated that the follow-up to twelve years will detect the more subtle defects (soft signs) seen in the minimally brain-damaged child which generally are not demonstrated by neurological and psychological tests. The subtle defects are often thought to manifest themselves through problems in school performance.

I. A number of papers were presented by members of the Section staff at scientific meetings. These include the following:

1. "Indicators of Morbidity in the Newborn", presented at the Congress of the Illinois Association of Maternal and Infant Health, Springfield, Illinois, February 18, 1965.

2. "Apgar Scores as an Index of Neonatal Morbidity", presented at the American Academy of Cerebral Palsy, New York City, December 19, 1964.

3. "The Relationship of Selected Characteristics in the Newborn to Neurological Morbidity During the First Year of Life", presented at the Society for Research in Child Development, Minneapolis, Minnesota, March 26, 1965.

4. "1964 Rubella Epidemic: Effect on Pregnant Women in the Collaborative Perinatal Research Study, Bethesda, Maryland", presented at the American Pediatric Society, Philadelphia, Pennsylvania, May 8, 1965.

5. "Toxoplasmosis: Serological and Clinical Studies of 23,000 Pregnant Women. A Report from the Collaborative Perinatal Research Study", presented at the Society for Pediatric Research, Philadelphia, Pennsylvania, May 6, 1965.

II. PROBLEMS:

The diagnostic summary to be designed at seven years presents several problems. This will necessitate collaboration between the pediatricians,

neurologists, psychologists, and speech, language and hearing examiners. To get these various disciplines to work productively in a collaborative effort will pose a real challenge to the leadership of the Section. Among the collaborators, the diversity of thought within a single discipline is great; when multiple-disciplines are brought together, the diversity of thought is compounded.

There are increasing commitments to data analysis which have placed additional demands on the Section staff.

III. PROPOSED FUTURE OBJECTIVES:

In the early part of fiscal 1966, the PED-12 backlog intake will be checked and the forms will be coded for the data file. Major emphasis of the Section during fiscal 1966 will be directed toward the introduction of the seven-year examination as of January 1, 1966. This will necessitate planning workshops and site visits for introduction of the form and manual to the collaborators. Data input of pediatric protocol will reflect a gradual decrease through the year of the nursery set (PED-1 through PED-8), and of the PED-10 (Four-Month Examination) following the cessation of obstetrical input. We anticipate a steady flow of PED-11's, PED-12's, PED-20's and PED-29's, and in addition will start receiving the PED-76 examination. Work must progress on the development of the diagnostic summary at seven years. This summary will include the Seven-Year Pediatric-Neurologic Examination, the Psychological Examination, and possibly the Speech and Hearing Examination at age eight. It will also include all the data in terms of historical events and documented medical events that are reported on PED-20 and 29 from age one to age seven or eight. All this material will be summarized on a precoded form similar to the PED-12. The planning of this protocol will require the intense participation of the Section staff and a group of consultants to the Perinatal Research Branch. There will be continued planning and discussion regarding the follow-up to 12 years, especially in terms of ways to gain maximum amount of knowledge within the boundries established for this follow-up. The Section will continue to participate in data output, subject to the limitations imposed on the entire Branch by inadequate funding for computations. The Section will make every effort to facilitate and encourage data analysis by the collaborators and it is hoped that the output in this area can be increased in fiscal year 1966.

IV. REMARKS:

Personnel: During fiscal year 1965, our temporary part-time neurologist became permanent and increased the time on the job from 4/8 to 5/8. Two of the statistical clerks in the PED Editing Unit left the Section and it was necessary to recruit and train two replacements.

Space: During the past fiscal year, the Section has moved into new quarters with approximately the same amount of space. While the quarters in the new building were not designed specifically to meet the Section's

needs, they are a marked improvement in that they are clean, well-lighted and generally pleasant. The new quarters are appreciated by members of the Section staff. With the addition of professional and nonprofessional members to the Section, it is anticipated that additional space will be required.

C. SECTION ON BEHAVIORAL SCIENCES
Report for period July 1, 1964 through June 30, 1965

SUMMARY OF SCIENTIFIC OR PROFESSIONAL ACCOMPLISHMENTS

During this period, 7100 8-Month Psychological Examinations were received at PRB and a total of 4000 records were coded and processed. Further editorial experience with this examination indicates that the overall quality of incoming records is excellent and that there are no problems concerning modification or revision of scoring existent in this examination. In fact the same conclusion was reached in evaluation of the quality of the records conducted during the period of January 1, 1963 to June 30, 1964, as indicated in the previous annual report. There are continuing requests for data from this examination in conjunction with new studies initiated by COLR obstetricians and pediatricians, both from the Perinatal Research Branch and the individual Collaborative Institutions. It is anticipated that, as the Collaborative Project continues, there will be increasing references made to the data collected on this examination with specific emphasis placed upon mental scores, and fine and gross motor scores. Qualitative aspects of the data, as they are represented in the Behavioral Profile will also receive increasing emphasis.

The 3-Year Speech, Language and Hearing Examination Further modifications of this examination, adopted in 1962, were completed during this period and no further revisions are being contemplated or needed. Editorial evaluation of incoming scoring records indicate that the efforts expended in the form of training workshops are beginning to pay off and that there is a further decrease in the number of errors noted in these records.

A system of quality control consisting of editorial evaluations and recommendations to the Collaborative Institutions, wherever disparities between the manual and application of the instructions to scoring records are found, is currently being carried out on an on-going basis. During this period, 4200 3-Year Speech, Language and Hearing Examination records were received at PRB, and a total of 5600 records were coded and processed. Several members of the Speech, Language and Hearing subcommittee served on ad hoc committees of the 7-year and 12-year follow-up task forces in an effort to determine the appropriate time for the administration of the Speech, Language and Hearing Examination, as well as its scope. Further coordination with psychological subcommittees and pediatric-neurological subcommittees charged with the development of the 7-year examinations was also successfully concluded by the Acting Head of this Section and a representative of the Speech, Language and Hearing subcommittee from the University of Minnesota.

Currently the Section on Behavioral Sciences is engaged in reducing the backlog occasioned by revisions in the manual and scoring procedures. Additional efforts are being made in the form of development of a new Speech, Language and Hearing Examination for administration to bilingual

or Spanish-speaking children at two of the Collaborative Institutions in New York; this has been completed and the Spanish version of the Speech, Language and Hearing Examination for the 3-year old is currently being administered to the Puerto Rican, Spanish speaking cases of the Study population. There has been partial resolution of the problem of obtaining qualified personnel in that further emphasis in the form of consultation with individual Collaborative Institutions has resulted in a partial reduction in the number of unqualified personnel charged with the administration of the 3-year examination.

The 4-Year Psychological Examination During this period, 4900 4-Year Psychological Examination records were received at PRB and a total of 4500 were coded and processed. Minor changes in administrative procedures outlined in the manual as well as scoring procedures were introduced during this period and backlogging of records for this examination have pretty much been cleared up. No further operational studies of the 4-Year Psychological Battery are contemplated since termination of the previous operational studies in February, 1964. It is anticipated that as data accumlate on this examination study proposals relevant to this area, and particularly in relation to the 8-month psychological in connection with the predictive effectiveness of the latter, will be forthcoming.

Maternal Intelligence During this period further methodological studies, five in all from five individual Collaborative Institutions were completed, concentrating on efforts to determine whether the SRA was a suitable test of Maternal Intelligence for use in the Collaborative Project. Results of these five studies indicate that the SRA was suitable for use in the Collaborative Project, with special provisions regarding the administration, scoring and interpretation. Specifically the psychology subcommittee, representing the committee of chief psychologists, recommended that raw scores and IQ scores be specified in the form rather than classifications of intelligence and that proper testing conditions and facilities be assured. Further recommendations concerned the use of the test for research purposes only with the specification that clinical evaluation of the intellectual status of the individual mother were not to be made on the basis of this brief examination, but that the results could be used for epidemiological purposes relevant to the study objectives.

A general presentation of the results was made to Project Directors of the Collaborative Project, and the SRA Non-Verbal Test of Maternal Intelligence was finally included as the instrument of choice and voted upon affirmatively by the Project Directors Committee on January 12, 1965. Copyright clearances were obtained from the Science Research Associates for permission to adapt the form for the Collaborative Project. The SRA manuals and forms were distributed to the Collaborative Institutions during the early part of March, 1965.

The Collaborative Institutions involved in the methological study on the validity of the SRA, utilizing the Wechsler Adult Intelligence Scale as criteria, were Providence Lying-In Hospital, New York Medical College,

Charity Hospital, University of Buffalo, University of Tennessee. Each of these Institutions collected thirty cases, testing mothers on both the SRA Non-Verbal Test as well as the Wechsler Adult Intelligence Scales. The data were transmitted to the Section on Behavioral Sciences for analysis and interpretation, resulting in the dissemination of five individual research reports on the validity of the SRA.

The 7-Year Psychological Examination Following completion of its review of the literature with the intent of obtaining suitable measures for the 7-Year Psychological Examination, the psychology subcommittee met again in October 1964 to construct the test outline and select the most promising measures for filling in this outline for the 7-Year Psychological Examination. Further delineations of the objectives of the examination were accomplished at this meeting in October 1964, and approval of examinations for each dimension of the examination was set up. Coordination regarding timing and content of the psychological examination was accomplished by two members of the subcommittee in meeting with a representative of the Speech, Language and Hearing subcommittee. As a result of these meetings, the examinations originally considered for inclusion in the 7-year psychological were eliminated, specifically those concerned with auditory memory, etc. in order to eliminate areas of duplication.

The Section on Behavioral Sciences then made arrangements to recruit selected and expert outside consultants to review the draft battery assembled by the psychology subcommittee on the basis of its extensive research and review of the literature. The subcommittee met with these outside consultants on January 18-19, 1965, along with representatives of the pediatric-neurological 7-year examination subcommittee to furnish information on any possible overlap between the 7-year pediatric and the 7-year psychological examinations. Extensive discussions were conducted between the psychology subcommittee and these outside consultants of national renown in the area of testing of exceptional children. Recommendations of the outside consultants were incorporated into the draft 7-Year Psychological Examination, and plans were made for holding a meeting of the committee of chief psychologists subsequently.

The Section on Behavioral Sciences along with individual members of the subcommittee prepared manuals and scoring procedures for the draft 7-year Psychological Examination in preparation for the meeting of the chief psychologists. The meeting was held in January 1965. The recommendations of the subcommittee and outside consultants were presented to the chief psychologists at a meeting chaired by a member of the Section on Behavioral Sciences. Each of the protocols suggested by the subcommittee and outside consultants was voted upon, resulting in a final battery which will be undergoing trial administration during the latter part of this year. Further analysis of the results of this trial administration should result in further additional streamlining of the examination battery within the time allotted for administration of this test in the Collaborative Project. Provisions have been made to collect at least 10 cases at each Collaborative Institution involving administration of the complete battery to determine

the time required for administration of the battery, objectivity of scoring and reactions of the examiners and examinees to the battery in terms of any problem areas that might arise.

8-Year Speech, Language and Hearing Examination In November 1964 and January 1965, the Perinatal Research Branch and the Speech, Language and Hearing subcommittee developed plans for the construction of an appropriate 8-Year Speech, Language and Hearing Examination for the Collaborative Project. The specific objectives of the 8-Year End-Point Examination and a draft examination outline were developed by members of the Speech, Language and Hearing subcommittee in cooperation with a member of the Section on Behavioral Sciences. Coordination with members of the pediatric-neurological 7-year subcommittee as well as the psychology subcommittee was carried out to avoid duplication or overlap between testing areas. At the meeting in January 1965, the Speech, Language and Hearing subcommittee developed a comprehensive examination outline and started developing materials and test contents for the 8-Year Speech, Language and Hearing Examination. PRB acted as a coordinating center for final evaluation of the testing materials and plans to make arrangements to have one of the members of the Section on Behavioral Sciences to visit each of the members of the subcommittee to assist in the development of the test contents. Additional planning for the 8-Year Speech, Language and Hearing Examination will involve a review of the 8-year battery by expert consultants who will be requested to furnish their comments and recommendations at a formal meeting scheduled for the future. In summary, the final steps in developing this examination involve the following: 1) assembly and development of administration instructions, scoring procedures, and coding procedures for the 8-year examination; 2) submission of the draft of the 8-year examination to outside consultants for further recommendations, modifications and revisions or additions; 3) presentation of a draft battery with the modifications and revisions made by consultants to the committee of chief Speech, Language and Hearing specialists; 4) pre-trial administration of this battery to determine suitability of time limits, objectivity of scoring procedures and reliability of observations.

II. PROBLEMS

A. Recruitment of Professional Personnel

In view of the influx of records on the 3-Year Speech, Language and Hearing Examination and the 4-Year Psychological Examination, the additional statistical clerk recruited in March 1964 has been fully trained, and the Section is in the process of reducing a considerable backlog to more manageable proportions. Several individuals during this period were interviewed for a position opening as a SLH specialist in the Section on Behavioral Sciences. As yet, the position has not been filled although there is a definite need to fill this position in order to meet Section objectives. Inspectional site visits as an

additional measure for quality control and evaluation of the data have not been accomplished adequately because of the shortage of professional personnel in the Section.

B. Space Facilities

The move to the WISCON Building from the Robin Building provided the Section on Behavioral Sciences with sufficient space for effective records maintenance. This no longer constitutes a major problem in terms of data handling.

III. PROPOSED FUTURE OBJECTIVES

A. One of the proposed uses of the 7-Year Psychological Battery is the identification of specific learning problems of those children considered to be mildly retarded, that is with I.Q. ranging from 50 to 75 especially upon entrance into school at grade 1, 2, or 3. The 7-Year Psychological Examination at present incorporates a short test of achievement as well as a global measure of I.Q. and should provide information which can be correlated with the child's performance in the follow-up phase now being considered for the Collaborative Project at 12 years of age.

B. Additional research objectives would involve the following:

1. Identification of specific problems these children have (reading or writing deficiencies, etc.) and the relationship these problems may bear to data collected on these children during the first 7 years of the Project's existence.

2. Identification of specific deficiencies uncovered in earlier Speech, Language and Hearing Examinations in relation to the psychological data obtained on the children as well as performance on the 8-Year Speech, Language and Hearing Examination. In view of the lack of explicit studies concerned with the development of language in the mildly mentally retarded child, this could well be the base point for further follow-up and evaluation of the child, possibly in connection with the use of various types of therapeutic techniques.

3. Research studies designed to determine the relationship between performance on the 8-month psychological in relation to the performance of the same child on the 3-Year Speech, Language and Hearing Examination as well as the 4-Year Psychological Examination. Research objectives in this phase would be directed to the development of effective predictors of aberrations as they would show up on this examination based upon evaluation of the child's mental, motor and behavioral performance on the 8-Month Psychological Examination. An effective predictor of future problems and

difficulties in the child's life, e.g., the 8-Month Psychological Examination, would be of great benefit in early identification and treatment of aberrant children.

4. In the 12-year follow-up extension of the Study, the Section on Behavioral Sciences anticipates being called upon to assist in the development of appropriate instruments for the evaluation of the child's academic progress, identification of aberrations in personality, behavioral problems, etc. As part of this follow-up, integration of the data already collected on Study Children, and the development of appropriate procedures for accomplishing this, is also anticipated as a future task of the Section.

IV. REMARKS

Needs

With the development and implementation of the 7-Year Psychological Examination and the 8-Year Speech, Language and Hearing Examination, it is anticipated that at least one perhaps two additional statistical clerks will be required for processing of the data on to punch cards and subsequently on to tape. In addition one professional Speech, Language and Hearing specialist and an additional behavioral scientist maybe required to handle the additional workload imposed by the implementation of these two examinations.

Additional space may also be required for files and records in terms of the increased workload.

The need for professional personnel to carry out the special site visits will also be increased since this is a routine on-going operation.

Personnel

As cited in above paragraph A

Travel

Annual meeting of professional associations of behavioral scientists as required. Attendance at meetings at which professional papers will be delivered.

D. SECTION ON INFECTIOUS DISEASES
(July 1, 1964 through June 30, 1965)

I. Summary of Scientific or Professional Achievements

A. Large-scale Serological Testing

Three types of studies are being conducted:

1. Broad serological approaches to identify the viruses which are infecting the study population.

Over 40% of the study patients were exposed to rubella during the nationwide 1964 epidemic. A new, rapid complement fixation test was developed for rubella and is available for current investigations. Serological tests are being applied as rapidly as possible to identify the patients who were at risk and those who were infected.

Over 22% of the study population tested has evidence of infection with at least 1 of 100 viruses tested. Over 5% of the study population showed antibody rise to Salivary Gland Virus, the virus that causes cytomegalic inclusion disease. Furthermore, 2% of the population had initial experience with Salivary Gland Virus during the pregnancy. Over 35% of the study population has had experience with Toxoplasmosis.

2. Intensive studies of important infectious agents.

Intensive studies of rubella reveal acute rubella in the mother and chronic infection of the fetus and child. These children are infectious.

Toxoplasmosis antibody was detected in 38% of 23,000 pregnant women tested. Markedly elevated titers were found in 2% of the specimens and 0.2% showed evidence of infection during the pregnancy. This data is being correlated with the pregnancy outcome. The information is now being analyzed with computer techniques.

3. Selected studies of pregnancies with abnormal outcomes and matched controls.

Selected studies of abnormals and matched controls have revealed several viruses which occur in significant frequency in patients with abnormal pregnancy outcomes. These are being investigated further.

B. Research on the development of antigens for use in the study of viral infections of pregnant women in the Collaborative Perinatal Research Study has resulted in the production of prototype quantities of 100 antigens.

C. A total of over 57,186 blood samples were submitted from July 1, 1964 through March 31, 1965, making a total of 263,182 on hand. The quality and quantity of the samples are over 90% satisfactory. This includes sera received from the study under the direction of Dr. Margaret Jones of the University of Southern California in Los Angeles and Dr. Paul McCallin, Kaiser Permanente Hospital, Honolulu, Hawaii.

D. Studies in Experimental Animals

1. Pregnant monkeys in Puerto Rico and Bethesda, Md.

Studies of pregnant monkeys infected with rubella virus were conducted in collaboration with the Laboratory of Perinatal Physiology, NINDB, San Juan, Puerto Rico. Animals were inoculated early in pregnancy. These showed evidence of infection. Some evidence was found for transplacental infection. Additional studies with squirrel monkeys showed similar findings.

2. H1 and H3 viruses and Congenital Malformations.

The H1 and H3 viruses were confirmed to produce malformations in experimental animals. Studies of patients in the Collaborative Study, however, did not show a significant association between these viruses and human disease.

3. Ferrets were found to develop experimental rubella when inoculated by the intranasal route. The drug Amantadine was found to moderate the infection.

E. Isolation of agents and Chromosome Studies

1. Rubella was isolated from more than 60% of fetuses obtained at therapeutic abortion for maternal rubella. Two patterns of recovery were found - disseminated and localized. Virus persisted in children with congenital rubella for at least 6 months but not 5 years.

2. A laboratory was prepared for chromosome studies.

F. Volunteer and Vaccine Studies

1. Several studies were conducted with volunteers at the Petersburg Federal Reformatory. Intradermal inoculation of rubella virus did not result in a skin reaction but clinical rubella occurred at the termination of the incubation period.

2. Epidemics of rubella were investigated in collaboration with the Arctic Health Research Center. These studies provided greater understanding of the infectiousness of rubella and the effect of gamma globulin on the prevention of rubella.

3. A small lot of rubella convalescent gamma globulin was prepared, and is being studied in Hawaii in a controlled investigation of the efficacy of gamma globulin for rubella.

4. The "Committee on Rubella Virus" was established and reviewed the current data on rubella. Areas which require investigation for the development of a vaccine were studied.

II. Problems

A. Lack of Funds for Materials Necessary for Studies

Because of the reduction in the research and Development Contract funds for virus reagents this year, it has not been possible to prepare many of the important antigens for testing. For example, only a few milliliters of CF antigen for rubella could be prepared. No cell pack antigens for the Adenoviruses, Salivary Gland Virus, or Herpes Simplex could be provided.

B. Insufficient Personnel

We are severely limited by the lack of sufficient positions for technicians, a secretary, Serum Center personnel, and statistical clerks. A minimum of six additional positions are necessary for efficient utilization of serum and antigen. This is particularly necessary because an increasing percentage of sera have demonstrated marked deterioration. Only through efficient utilization of procedures which are available will it be possible to obtain information concerning the exposure of the patients to viruses and the importance of this exposure to the production of abnormal infants.

C. Variable Antigenic Preparations

It is apparent that the biological systems involved in producing the antigens are frequently not reproducible. Frequent changes in procedures, changes in tissue or special methods of concentrating antigenicity are necessary to obtain a new lot of antigenic material. In other cases anticomplementary effects must be eliminated or the antigens may have to be remade. The production of antigens requires individual research and development.

D. Limited Studies

Two factors which are limiting testing at the present time are the incomplete serum collection during the first two years of the study, which resulted in numbers of patients for whom few of no specimens are available; and incomplete identification of abnormal pregnancy outcomes which restricts identification of serum specimens for serological testing.

III. Proposed Future Objectives

A. Additional Personnel

Every attempt will be made to obtain the six new positions in Fiscal Year 1966 which are absolutely necessary for our study. Two of these positions will be for laboratory technicians for emergency testing of sera which are undergoing loss of activity; two positions will be for assistance to unpack and make available the back log of over 1 year of serum collection (160,000 vials). Two positions will be used for analysis of the backlog of test data and secretarial assistance to correlate with data being punched from the Obstetrical and Pediatric records.

B. Antigen Production

Additional funds for reagents will be sought so that antigens for rubella, salivary gland virus, varicella and new viruses will be prepared and incorporated in the testing program. Several new bacterial and protozoal antigens will be added to the routine testing of the study sera.

C. Antisera Production

Prototype sera will be prepared for 10 viruses for control testing.

D. Large-scale Testing

Serological studies involving 100,000 specimens from pregnant women in the Collaborative Perinatal Research Study will be conducted. The tests will include antigens for Flu A, Herpes Simplex, Mumps, Salivary Gland Virus and Rubella. Increased investigations of cross reactions with serological tests will be conducted. Supplemental sampling of the study population will be necessary to include patients who register early in the first trimester of pregnancy so that documentation of virus experience in this period will be possible.

E. Cytogenetic Studies

A new laboratory for cytogenetic studies will be opened to work in conjunction with the infectious disease studies of abortion. This laboratory will include one professional, two technicians, and an animal handler.

F. Rubella Study - Collaborating Hospitals and Cooperating Institutions

Collaborative studies on the epidemiology of rubella will be pursued with the Study Hospitals and with Dr. Margaret Jones (U.C.L.A. - Kaiser Hospital, Los Angeles), as well as with Dr. Paul McCallin (Kaiser Hospital, Honolulu, Hawaii).

G. Specimen Collection

Specimen collection will be expanded to include tissue samples from placentas, abortuses, at the Kaiser Hospitals in Los Angeles and Honolulu, so that the isolation of viral agents may be utilized to confirm and extend the serological findings and simultaneous cytogenetic studies will be performed. Special fetal specimens will be studied from patients with rubella.

H. Experimental Animals

Further investigations will be conducted with experimentally infected pregnant and nonpregnant animals to provide direct information on routes of infection, pathogenesis, and possible methods of prevention of defects in the fetus due to infectious agents. Experimental animal studies will continue in pregnant monkeys at the Laboratory of Perinatal Physiology, San Juan, Puerto Rico, and with pregnant monkeys obtained under contract arrangements. Viruses under study will include rubella, mumps, and salivary gland virus.

I. Gamma Globulin Study

The efficacy of the use of gamma globulin for the prevention of rubella will be tested using titered gamma globulin in controlled studies in Honolulu, Hawaii.

J. Vaccine Studies - Rubella Virus

Further studies on inactivated and attenuated vaccines for rubella will be pursued. These will include animal inoculation of vaccine materials to determine antigenic potency as well as possible administration to volunteers using safety tested material.

I. Summary of Scientific or Professional Accomplishments

A. Automated Biologic Image Processing

Collaborative research with the Artificial Intelligence Group of the National Bureau of Standards on the plans and specifications for a general purpose device began on a formal basis on July 1, 1964. The objective of the general purpose device is to provide for the conjoined human-computer analysis, synthesis and description of biological images. The results of the work thus far with respect to the general purpose device itself are summarized in the attached individual project report. (Serial No. NDB (CF)-65 PR/P 1278)

Previous attempts at biologic image processing, particularly of photomicrographs, has stressed the measurement of various parameters and may be characterized as a classical statistical approach to the problem of image characterization and pattern recognition. The general purpose device represents an alternative approach which replaces succinct statistical characterization of images with more elaborate articular description of the same images. The description is articular in the sense that the structure of the original images is partially preserved. Elaborate description is necessary to preserve structural data about the images. To process such elaborate descriptions, it is of course necessary to use description vehicles capable of machine processing. These vehicles turn out to have properties related to those of natural languages. Thus, linguistic description of biological images is employed to abstract structural properties for machine processing. The linguistic description assumes two forms. The first of these is textual and is represented by the typed input of the operator. The other form of description which develops is also of a linguistic character and is best characterized as being pictorial. The fact emerging from the work on the general purpose device is that there are strong interrelations between the textual and pictorial descriptions of a biologic image. The ITSELF system, our term for the general purpose device, is being planned to have the capacity for deep analyses of images with much structural complexity as well as having the by-product capacity for such techniques as sketching by machine and generation of description of images by the machine.

At a practical level, in addition to the accomplishments detailed in the individual project report, two accomplishments of great immediate significance for the work of the Section on Pathology should here be emphasized. The first of these is the demonstration of feasibility of machine generation of three-dimensional reconstructions of optical or microtome serial sections. This means that celloidin sections of 50 microns or more thickness may be examined and these largely independent of the staining techniques that have been employed in the preparation of the slide. Thus, sections such as those prepared by

Dr. Yakovlev for the Collaborative Project will eventually be amenable to machine analysis. The optical difficulties occasioned by such large and thick sections are being partially obviated, if not completely so, by a special low power objective of high numerical aperture which is being developed by Carl Zeiss at our suggestion.

The investment of research effort in the general purpose device has yielded a second set of results which, when taken together, now make a special purpose neuron counting system feasible and practical. These results which may best be characterized as logical in nature, comprise those obtained by Dr. George Moore in the expansion of his programs for the large Pilot Computer and certain image differentiating techniques developed by Mr. Russell Kirsch which are essentially algorithms for edge identification. Taken together, this represents the logical design for the processing of information from a scanner (such as the one already in existence at NBS). By suitable logical manipulations, this special purpose neuron counter should be independent of the staining method employed. It is proposed during the following 12-month period to present a practical demonstration in the form of simulation on a general purpose computer of the characteristics of such a special purpose device. This latter will be accomplished independent of the National Bureau of Standards group since the agreement calls for their concentration on the general purpose device to the exclusion of all others (see below).

1. Special Purpose Device Work

During the Third Workshop on Cell Counting and Discriminating Instrument Development in January 1964, several special purpose device configurations were presented as potential neuron counting devices (see the previous annual report). Of the three major systems presented, one of them, the CELLSCAN system, is the subject of considerable renewed activity. This, however, is activity directed towards its utilization as an identifier of white blood cell types in actual smears. The other two commercial projects are inactive as far as can be determined with respect to the neuron counting problem.

The above noted dividends of the research on the general purpose device would suggest the extreme desirability of early accomplishment of the demonstration project noted above. This requires the accessibility either of a medium sized hybrid computer, e.g., the CDC 3100 Hybrid expected at NIH during the year 1965, or alternatively, the small general purpose computer with extensive analog to digital conversion facilities such as the LINC. Efforts are being made to obtain the use of either one of the above-mentioned computers, for the present through normal channels.

The LINC would also permit increased support activity with respect to special device projects being carried on elsewhere, since with the LINC an automatically programable microspectrophotometer is possible. This role is essentially to fulfill the mandate of the Second and Third Workshops for Cell Counting Instrument Development as well as to comply with the instructions of the Director of NINDB to serve as a central reference laboratory for biologic image processing activities. This mission is to provide "the quantitative biologic data to extra- and intramural biologic image processing projects not otherwise obtainable." A detailed prospectus demonstrating the utility of such a functionally programable microspectrophotometer was prepared and accompanies a formal request for the computer component of such a device.

Pending the realization of these plans, Dr. Jack H. Carleton has completed an investigation in cooperation with Dr. Eli M. Nadel of the National Cancer Institute on "Feulgen DNA Content in Guinea Pig Leukemia Using CYDAC as a Cytophotometer," which showed this instrument's capability to provide early evidence of abnormal cells. This study is being reported at the Second International Congress of Exfoliative Cytology, May 1965. It has also permitted him to use this and other instrumentation on a current investigation to study the cytophotometric staining properties of gallocyanin in the central nervous system. These ongoing studies have been devised with Mr. John Laskey of our laboratory to establish the most reliable gallocyanin technique for future utilization in quantitative cytophotometric techniques as applied to the central nervous system.

An investigation to determine the fading rates of feulgen stained central nerve cells stored under usual conditions was initiated and completed by Dr. Jack H. Carleton and Dr. Charles Spencer of Hagerstown, Maryland. The experiment has shown that touch imprints of mouse cerebral cortex stained by a modified feulgen technique, mounted in three different medias, and stored under identical conditions in the dark at room temperature, have less than five percent loss of absorbence over a period of 78 days. The results of this study are being prepared for publication and offer valuable information in setting up future investigations, especially where comparisons of transmission values at long time intervals are mandatory.

B. Coding and Data Processing

1. Systematized Nomenclature of Pathology

The Systematized Nomenclature of Pathology sponsored by the College of American Pathologists was published in its first edition in January 1965 with the Head of the Section on Pathology as one of the 18 co-authors. (Lipkin, Lewis E., Co-Author: Systematized Nomenclature of Pathology. College of American Pathologists, Committee on Nomenclature and Classification of Disease.)

Employing this coding system, the analysis of Path-III has been started. In addition to an overall print out of frequency of autopsy diagnoses, breakdowns in tabular form have been prepared. These, thus far, consist of tables showing the effect of birthweight, type of event, race and sex on the incidence of various diagnoses.

2. Study of Predictors of Organ Weight

The quantitative data present in Path-III reports is now also on punch cards. A study of the statistical validity of various predictors of organ weight is now in the preliminary stage of processing. Such predictors would include body weight, crown-rump length, crown-heel length and placental weight. The procedure planned is that of multiple regression analysis. A special program to deal with such data has been written within the Section on Pathology and is presently being debugged. This project is still at too early a stage to warrant an individual project report.

3. Brain Processing

There are no essential changes in procedures and methods beyond those reported in the previous annual report.

C. Laboratory Procedures

At the beginning of the reporting period, a small electron microscope was transferred to the Section on Pathology from the Laboratory of Perinatal Physiology. An Electron Microscopy Laboratory has been set up and the senior histologic technician has been completely trained as an electron microscopy technician. The electron microscope is under the immediate supervision of Dr. Jack H. Carleton who has begun to explore the feasibility of an electron microscopic study of the healing of a cerebral wound.

D. Special Studies

1. Hyaline Membrane Study

A detailed study of the incidence and histologic characteristics of hyaline membrane disease in the project autopsy population was completed by Dr. Luz A. Froehlich and Dr. Toshio Fujikura.(NDB (CF) 65 PR/P 1279)The relationship of this entity with basic factors such as race, neonatal mortality rate, birthweight, postnatal age and associated pulmonary histologic findings were analyzed. The manuscript is presently with the Publications Review Board.

2. Umbilical Cord Inflammation Study

This study previously reported was begun by Dr. Luz A. Froehlich and has continued, and the initial stage of collection of 1,000

such specimens has been completed. At the time of writing, the results are being subjected to detailed data analysis. It is obvious without such analysis, however, that there is very good correlation indeed between the incidence of inflammation as detected from smears with that detected as a result of examination of histologic sections. Data analysis which is proceeding involves not only morphologic but clinical correlations. (Serial No. NDB (CF)-65 PR/P 1280).

3. Effect of Hyperoxia on the Offspring of Pregnant Rhesus Monkeys

A pilot study is being undertaken by Dr. Toshio Fujikura in collaboration with Dr. Ronald Myers of the Laboratory of Perinatal Physiology in Puerto Rico. Dr. Myers has generously made available four pregnant monkeys, which Dr. Fujikura exposed to 100 percent oxygen at normal atmospheric pressure for a measured length of time. The purpose is to ascertain whether material hyperoxia can produce retrolental fibroplasia and other neurologic abnormalities. Dr. Myers has just reported that all four have delivered and thus far the neonates show no obvious physical and neurologic abnormalities. Since the exposure was done late in pregnancy, Dr. Fujikura plans to expose another set of pregnant monkeys early in pregnancy.

4. Corneal Graft using Human Amnion

There is a close resemblance histologically and physiologically between the cornea and the amnion. The remarkable similarity of their collagen fibrils are shown by electron microscopic examination. These are the important facts supporting the possibility of corneal amnion graft. A pilot study is underway between Dr. Fujikura in collaboration with Dr. William Green of the Ophthalmology Branch, NINDB, using human amnion grafts on rabbits. If successful, the merits of this experimental study would indeed be great because of the easy availability of the amnion.

E. Activities with Non-Project Material

1. Medical Neurology Branch, Clinical Center

The program of neuropathologic support of the Medical Neurology Branch at the Clinical Center is continued on an unchanged basis from that noted previously.

2. American Academy of Cerebral Palsy

The agreement with the American Academy of Cerebral Palsy although formally in existence has not yielded material of very great interest. In December 1964, Dr. Preston Rob assumed the position of Chairman of the Brain Registry and Research Committee of the American Academy of Cerebral Palsy. Subsequent conversations

with Dr. Rob have clarified the position of the Academy and have also indicated guidelines for replanning of the brain registry activity. The objectives of these plans are largely oriented toward increasing the amount of information obtained from the limited amount of neuropathologic man hours expended on the case material which because of inadequate case selection has been relatively low. There is every reason to expect that the new guidelines proposed by Dr. Rob will favorably alter this balance.

II. Problems

A. Data Processing Facilities

The need for access to a medium sized computer or a laboratory type instrument such as the LINC has been previously detailed. In addition to providing for the image processing requirements of this laboratory, the turn around time for experimental analysis and experimental design construction would be more favorable if such computer facilities were immediately available. A third advantage would be in the utilization of display subsystems as possessed by both computer systems named. Since such facilities as present in the LINC would also be available to the Section on Statistical Analysis, the possession of a LINC in this laboratory would constitute a multiple advantageous situation.

B. Path-I and Path-II Forms

There are approximately 37,072 Path-I and Path-II forms which are still uncoded. Early in the Study about 18,000 Path-I's and Path-II's were coded and punched but with inadequate professional editing, partly because the responsibility of these forms at that time lay in a section other than Pathology. It is apparent that more highly reliable data can be obtained from these forms by carefully editing the unstructured comments. Presently, only one medical editor is available to edit these forms under supervision of the pathologists in the section. Temporary or part-time personnel are being recruited to assist in the editing of this large backlog and it is expected that this portion of the work will be accomplished within approximately four months.

C. Personnel

There has been essentially no change in the personnel requirements of this section as outlined in the previous annual report.

D. Space

The need for animal space has become even more acute with the active operation of the electron microscope. No solution to this problem is presently apparent. Productivity of the electron microscopic research program is being severely curtailed by the

lack of small animal facilities. The present small scale availability of primate facilities in Puerto Rico has partially taken care of the needs of the congenital malformation project. It is hoped that this may be expanded subsequently.

III. Proposed Future Objectives

A. General

The Section on Pathology will continue to concentrate on the studies of project material in ever-increasing detail. The availability of a special purpose neuron counting device would greatly speed the accumulation of microscopic data. It is hoped that the demonstration project mentioned above will lead to the acquisition of such a special purpose device, especially as it now presents no basic engineering difficulties.

B. Specific

1. Pursuit of the general purpose device along the lines previously established with particular emphasis upon areas such as machine generated three-dimensional reconstructions.

2. Pursuit of established research projects.

3. Establishment of a program of visiting project pathologists. This would entail visits by project pathologists for a period of from one week to six months to study specific items to be found in the slide banks now well established in the section. Pathologists at Collaborating Institutions having specific interests in morphologic problems which are amenable to study here at the central laboratories will be invited to conduct those parts of the study involving the microscopic examination of slides here in the central laboratories and offices. It is hoped that the tables generated as a result of data processing of the diagnostic card files will generate sufficient interest among project pathologists to provide impetus for such a program.

IV. Remarks

Personnel

New Professional Personnel

Carl M. Leventhal, M. D. (Neuropathology) *

Jack H. Carleton, M. D. (Neuroanatomy and Electron Microscopy)

*Will present a paper at the American Neurological Association, Atlantic City, New Jersey, June 15, 1965. C.M. Leventhal, J.R. Baringer, B.G. Arnason and C.M. Fisher: A Case of Marchiafava-Bignami Disease with Clinical Recovery.

Consultants

Robert Beare, Ph.D. (Linguist, NINDB consultant on biological image processing in its linguistic aspects.)

Transfers from Technical to Professional Category

Phyllis Gordon (Biologist)

Organization

There have been no essential changes in the geographically imposed division of the section into the fetal and placental pathology units and the neuropathology unit.

F. SECTION ON EPIDEMIOLOGY AND GENETICS
Report for the period July 1, 1964 - June 30, 1965

I. SUMMARY OF SCIENTIFIC AND PROFESSIONAL ACCOMPLISHMENTS

The Section has continued to receive, collect, coordinate and code information in the genetic and socioeconomic areas. At the same time it has expanded considerably its research activities so that for the first time it has been possible to use the collected data to the fullest extent that they could be handled by our limited research staff. The research projects which were started last year were carried through and in addition numerous new projects encompassing a variety of genetic and socioeconomic problems and situations were initiated. A summary of these is given below.

The Section moved physically into new and spacious quarters in the Wiscon Building, in Bethesda. There is now adequate space for the professional and clerical staff and also for the storage of records. The present space division is flexible enough so that it can accommodate the ever expanding volume of files to its maximum anticipated growth.

GENETICS

The activities in the genetic area during the past year have been mainly directed towards the development and design of good genetic studies. Routine coding and processing of the incoming genetic forms has also continued. During this time about nine thousand forms, mainly GEN 5-8, have been received and an equal number coded, checked and sent for punching. Dr. Naylor, a population geneticist who joined this Section last July, has been instrumental in developing and designing the new genetic studies and has had the opportunity to do some of the programming himself. The studies which began during the last fiscal year and were reported in last year's annual report were carried through but very few have been completed because of lack of computer time. Apparently, although computer facilities were available to the Branch during the past year, most of the computer time was devoted to transferring the information from the existing cards to tape and making the necessary corrections. We are now informed that computer time is again available to process the approved studies.

The determination of zygosity of twins born to Study mothers continues. The objective of this operation is to determine the zygosity of all twins born to the Study mothers so that anyone wishing to engage in twin research may have the material available. Unfortunately, the Collaborating Institutions are derelict in obtaining the required finger and palm prints and blood samples from the twins. As a result, the number of twin pairs for whom zygosity has been determined is far

below the actual surviving number of like-sexed twins born to the Study. The study of major congenital malformations has already been programmed for multiple regression analysis and will be ready soon. A series of four studies has been designed to determine the role of genetic factors and the relation of age and parity in habitual abortion. Another study relates the prior pregnancy experience with Study pregnancy outcome. Dr. Naylor has been specifically interested in whether or not there is a genetic basis in neonatal reflexes and also in the effects of fetal loss due to Rh immunization, on family size. In addition, Dr. Naylor has tackled the problem of efficient test statistics for use with epidemiologic data.

The expanding genetic activities of the Section bring into focus again the necessity for establishing the cytogenetics laboratory which was recommended in last year's report. It also raises the problem of the proper analysis of our genetic material. Our views and plans regarding these matters will be developed in a later section.

SOCIOECONOMICS

In parallel with plans for genetic studies and genetic analysis of the material, plans have also been made for supportive socioeconomic studies. The project to develop a socioeconomic index, which was mentioned in last year's report, has been completed. The objective has been to develop an index which would categorize and describe our Study population and also compare our Study population to the United States population. The methodology used follows very closely that of the U. S. Bureau of the Census which develops scores for three major socioeconomic variables, education, occupation and income, averaged to give a composite index. These scores have been derived in two ways: first empirically, using the distribution of these variables in our own population; and second by applying the individual scores which the U. S. Bureau of Census derived for each variable, to our own population. These scores, and the resulting indices for every case in the Study, are now being put on tape and should be ready very soon for use by anybody who is interested in socioeconomic studies.

The study of the effect of several socioeconomic variables on birthweight is in its final stages of completion. It has been properly programmed and the results will shortly be forthcoming.

This Section has been instrumental in bringing together several interested Institutions at a meeting to discuss the feasibility of doing a socioeconomic and sociocultural study in depth. Although no definite decisions were taken, the need for such a study was felt by all. It was recognized, however, that at present the design and execution of such a study was beset by administrative difficulties for it appears that it cannot be considered as a part of the Core Study and funds for its support must be sought for elsewhere.

This Section, acting on a directive of the Project Directors Committee, developed a new form known as the FHH-9. This has been designed to bring up to date the socioeconomic and genetic information which was collected prenatally and it will be administered with the battery of tests at the seven to seven and a half years examination. Dr. Kathrine French of the University of Oregon has been instrumental in developing this form and the accompanying manual. The form was pretested at all Institutions and was found to work well, and to fulfill its purpose. The form was accepted by the Project Directors as a regular study document. It will go into effect around the middle of 1966.

During the period covered by this report the coding and punching of the SE-1 form was also begun. Around eleven thousand SE-1 forms have already been processed and will shortly be available for purposes of analysis. The problem of analysis which was referred to with a respect to the genetic data also applies to the socioeconomic material. This Section still does not have a sociologist on staff in spite of very active recruitment. The problem of analysis of the socioeconomic material is crucial and the proposal made by Dr. Sidney Goldstein that we open this material to selected graduate students in Sociology was favorably received and is discussed in a later section.

FIELD WORK

One of the increasing activities of this Section during the past year has been that of field work. It was mentioned in last year's report that as we develop special studies to answer specific questions we will have to depend more and more on field work. At the present time, our field activities are being carried out by only one field worker, Mrs. Vincent, but the anticipated increase of field work makes it imperative that we establish and train a field team to collect the needed special data.

During the past year Mrs. Vincent made the final rounds of several Institutions, especially on the eastern shore to retrieve as many as possible of the remaining cases of mothers who aborted and would otherwise have been lost to the Study. In another assignment, Mrs. Vincent, along with a team from the Obstetrics Section, exemplified the characteristics of good field work: swiftness and mobility. When the need arose to study as quickly as possible those mothers who were exposed to rubella, and their offspring, the team immediately moved to Boston where during one week-end went through more than fifteen hundred records to select those which were needed for the Study. Mrs. Vincent then moved from Boston to Providence where alone she reviewed about six hundred records in the span of a few days. The rubella study has been accomplished in time thanks to the mobility and swiftness of the field team and especially our own experienced field worker. Mrs. Vincent has also been devoting a lot of her field work time to the retrospective study of brain damaged children in the D. C. area which is conducted under the direction of Dr. Shakhashiri. This

requires personal contact and a lot of driving and foot work on evenings and during the week-ends but these techniques are the stock in trade of an experienced field worker.

A joint undertaking is now being planned by this Section, the Section on Infectious Diseases and the Institute of Child Care and Human Development, to do special studies of karyotypes of abortuses and malformed children. This undertaking will require intensive field work which this Section is expected to provide.

II. SPECIAL PROBLEMS

A very important if not critical problem now facing this Section, which will become more critical as time goes on, is the lack of trained professionals among the Collaborating Institutions to participate in the analysis of the genetic and socioeconomic material. Although the Collaborating Institutions have on staff to the Project, a plethora of obstetricians, pediatricians and psychologists, they do not have a single geneticist or sociologist. Dr. Myrianthopoulos has made repeated appeals to the Project Directors both during private conversations and in plenary meetings to consider seriously this need and their obligation to participate in the analysis of the genetic and socio-economic material. As matters stand now, it appears that this Section will have to bear the burden of analysis alone. Dr. Sidney Goldstein, our socioeconomic consultant, suggested that we open our socioeconomic material to selected graduate students in Sociology who would have the benefit of well collected material for theses projects, and at the same time we would benefit by having our material analyzed by competent and well supervised young professionals. The same can obviously be done with the genetic material. It appears to us that such an arrangement is workable and mutually beneficial and we recommend that it be implemented as soon as possible.

III. PLANS FOR THE FUTURE

It is anticipated that by the end of calendar year 1965 a sample of about sixty thousand gravidae will have been collected and further intake of cases will cease. It is appropriate, therefore, at this time, to shift our thinking from the data collection phase to that of data analysis. One of the most important readjustments which will have to be made in the make-up and operation of this and other Sections of the Perinatal Research Branch would be that of systematic conversion of positions, from semi-professional and clerical, which now constitute the majority in each Section, to professional and related technical positions. This will involve abolishing some semi-professional and clerical positions, retraining some of the more competent personnel to do professional or technical work, and recruiting for more well-trained professionals. This conversion will have to come about very slowly, and we have already taken the initial steps to accomplish it. We are now actively recruiting to fill two professional positions, for a sociologist and a cytogeneticist; we are taking steps to establish

a field team with at least three trained persons; and we are making plans for the re-training of some of our more promising auxiliary personnel to do professional work.

We must reiterate again that the genetic studies in which we are interested and which we are planning will depend to a large extent on the presence of adequate laboratory facilities. These various investigations are logical corollaries of the first phase of the Collaborative Project and they should be pursued without delay. Many strong pleas for the establishment of a cytogenetics laboratory were made in the past on our part and it appears that the prospects for establishing it this year are quite good. We are glad to realize that those charged with giving direction to the Project see the need for the establishment of such a laboratory and we hope that they will make the appropriate space available.

We also urged in the past that an immunological and biochemical laboratory be established in order to elucidate a variety of problems which will stem directly out of the preliminary analysis of the data. We mentioned that retrospectively, when a genetic abnormality has been verified in the offspring, certain biochemical determinations may be indicated in the parent. These would include tests for the recognition of the heterzygote. In cases where no satisfactory tests are available, constant attempts to detect biochemical differences should be made. It becomes more and more evident that we will be needing additional serological information for the projected genetic studies, such as blood types of the father of the baby and a variety of enzymatic tests, including an assessment of polymorphic properties of haptoglobins and transferrins. These biochemical determinations need not of course be performed in a central laboratory, located in Bethesda. Arrangements, however, must be made for the performance of all these tests either locally at each Institution or centrally, to serve a group of Institutions. The important thing is not where they should be made, but that they be made and that facilities be available for them to be carried out when the need and the situation arises.

Looking ahead to the fullfilment of the goals of this Project we urge that serious consideration be given to the establishment of these laboratories in the development of the second phase of our program.

IV. REMARKS

Personnel

The personnel of the Section on Epidemiology and Genetics consist at present of the following: Professional, Dr. Myrianthopoulos, Head, Dr. Naylor, Geneticist, Mrs. Vincent, Senior Nurse Officer and Field Worker, Miss Martin, Statistician; other: one secretary stenographer, one clerk typist, four coding clerks in the Socioeconomic Unit, one supervisor, three coding clerks and one part-time clerk in the Genetics Unit.

We are still actively recruiting for a medical sociologist or a developmental psychologist to help in the direction, design and analysis of socioeconomic studies. We have also started to recruit actively for a cytogeneticist to organize and direct a laboratory of cytogenetic studies. The prospects in this area are quite good and well trained people are available. Depending on the availability of laboratory space, it is anticipated that we may be able to bring a cytogeneticist into our program soon.

Activities of the Section Head

The Head of the Section, Dr. Myrianthopoulos, in addition to his formal duties, has carried out independent investigations, mostly continuations of projects which he started before joining the Perinatal Research Branch. Although these are not directly connected with the activities of the Branch, they are being described in the individual project reports since they represent work carried out by the personnel of the Section.
Dr. Myrianthopoulos has maintained his affiliation with George Washington University as an Associate Professor of Neurology and Director of the Genetic Counseling and Research Center. In this capacity, he gives a series of lectures in the Medical School and conducts genetic counseling upon referral by individual physicians. Dr. Myrianthopoulos is also a member of the faculty of the Graduate Program at NIH where he gives a course in Human Genetics.

Dr. Myrianthopoulos was invited to participate in the second Panhellenic Neuropsychiatric Congress which convened in Salonica, Greece, during the latter part of May 1964. Dr. Myrianthopoulos attended as an official NIH representative and addressed the Congress on the role of genetics in modern neurology. He also presented a paper on the genetics of schizophrenia. On his return trip, Dr. Myrianthopoulos stopped off in London, England and conferred with Professor Penrose of the Galton Laboratory concerning the genetic aspects of the Collaborative Project.
Dr. Myrianthopoulos attended the annual meeting of the American Society of Human Genetics which took place in Boulder, Colorado during August 1964 and delivered a paper on conduction volocity tests in Charcot-Marie-Tooth Disease. Dr. Myrianthopoulos also participated and lectured at various meeting and symposia throughout the country during this period.

Honors and Awards

In last year's report, a remarkable achievement in field work by a member of this Section was described. Mrs. Vincent was able to retrieve over 600 cases of women who aborted soon after they registered in the Study, before genetic and socioeconomic information was obtained, and for all intents and purposes were lost to the Study. Mrs. Vincent accomplished this feat practically

single-handed with exemplary dedication, thoroughness and accuracy. An important phase of the Collaborative Project was thus saved.

It was felt that such an achievement should be recognized and rewarded, and Dr. Myrianthopoulos recommended Mrs. Vincent for the Commendation Medal of the Public Health Service. The recommendation was approved and Mrs. Vincent was awarded the Commendation Medal by Dr. Masland in an appropriate ceremony.

Dr. Myrianthopoulos was elected to the New York Academy of Sciences.

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Naylor, A. F.: Comparisons of regression constants fitted by maximum likelihood to four common transformations of binomial data. Ann. Hum. Genet. Lond. 27:241-246, 1964.

Naylor, A. F.: Natural selection through maternal influence. Heredity, 19:509-511, 1964.

Naylor, A. F.: Dispersal responses of female flour beetles to larvae in a discontinuous environment. Ecology, in press, 1965.

I. Summary of Scientific or Professional Accomplishments

This year was one of considerable accomplishment for the Section on Systems Design and Procedures. It was the first year during the life of the Study in which substantial data processing facilities were available. These facilities were available from the middle of July through the end of the fiscal year. This permitted the first attempt at the construction of permanent complete data files and data processing systems in the Collaborative Project. All attempts in prior years were task-oriented in that the specific goal was the production of a specific group of studies or tabulations of material without regard to its continuity. No permanent arrangement could be made since no assurance of continuity for data processing existed. During the year a continuing effort was made to support, both with funds and services, the development of programming techniques at the Computation and Data Processing Branch, DRS, although these still are far from being complete. The services of the Computation Branch may not be fully available until sometime in 1966 or 1967 when it is hoped that both the new computers and new organization replacing the Computation Branch will be in existence. The Perinatal Research Branch, through SSDP, produced a complete data file consisting of approximately 2 1/2 million punch cards representing 91 different card formats in the current fiscal year. The data files were edited and checked for validity of codes, completeness, duplicates and missing cards. The system was developed not only for checking these cards and putting them into the data file, but for their maintenance and updating. This means adding additional cards to the file, making corrections and deletions and additional editing and checking of the data. The system includes cross-checks between files and the continued construction of new files as they become available in the Study. Several methods of constructing a working data tape file from the basic data file have been used and tested and the relative merits and objections to each one have been examined in order to minimize cost and promote efficiency. Complete frequency distributions for each of the files on tape have been programmed. Over two-thirds of these frequency distributions were completed by March, 1965, and were distributed to all the collaborating institutions. The balance will be completed and distributed by the end of the fiscal year. In addition, a series of special tabulations relating selected outcomes to events during pregnancy have been prepared as further means toward analysis of the data. It has already been demonstrated that the frequency distributions and cross-tabulations have considerable merit not only in their own right for understanding the data or understanding the relationships observed, but for the quicker development of more sophisticated studies. In a number of instances, they have proven invaluable in establishing the class intervals and the type of analysis to be used in a given study.

A Branchwide system has been developed for review and checkout of studies to insure their research quality and suitability for analysis. The Section participated actively in this development. A quantity of specific retrieval requests have been completed and a substantial number have been used in various presentations by collaborators of the Study during the fiscal year. Many of these have resulted in the production of specific papers published and/or presented at various scientific meetings.

A programming unit was established in the Section during the year to permit the Branch to use the software techniques developed for use on NIH computers and to permit utilization of services available under the Computer Sharing Service (NBS) for the Washington, D.C. area.

Two fully qualified programmers are currently employed, as well as a junior programmer, who has received considerable training, and a programmer-trainee. The unit has had considerable productivity relative to its size, but will have to expand considerably to meet its proposed goals.

The Section, as part of its own programming effort, has developed a retrieval system for drugs taken during pregnancy to serve as an evaluation screen and has expanded this screen to include similar reports from U. S. Navy hospitals facilities.

Various special review systems have been developed--particularly programs which permit the obstetrical summary events to be reviewed in detail against selected outcomes in the newborn period or for the one year neurological examination. Similar programs have been developed for review in detail of events reported on the nursery summary and the summary at one year of age.

While sample maintenance is considered the direct concern of each collaborating institution, and control and day-to-day evaluation of efficacy is at the local level, the Section has a coordinating and advisory function. A section staff member serves as liaison between the institutions and PRB, answers questions and aids in problem solving. As part of this function, on December 11, 1964, the Branch, at the request of the Project Directors' Committee, conducted a sample maintenance workshop. This workshop dealt with a number of common problems affecting each institution. Proposed solutions to these problems were presented to the subsequent Project Directors' Committee and most were adopted. These represented new problems occurring because of the continued follow-up of children, clarifications of existing procedure to insure uniformity of application, or revisions of procedure to improve effectiveness.

Tabulations examining effectiveness of sample maintenance, as measured by completed forms submitted to PRB, were also presented at the workshop.

These were derived from reports from the record inventory system of the Branch. At present the outputs from the inventory system consist of various tabulations of data concerning the admission of gravida to the Study, and characteristics of terminated pregnancies, produced on a monthly basis and reports on submitted forms for the current year and past two years. At present, using the data file we have prepared an input record containing all the forms received and processed in prior years. Sometime before June 30, 1965, this file will be added to the inventory system. At that time, complete reports on submitted forms for all years of the Study will be available on a regular basis.

During the year, the Section has further consolidated or centralized most of the coding and filing units of the Study so as to minimize communication problems and produce more economical means of processing and storing Study forms.

Coding in most areas has progressed as expected and has continued at a steady rate. It is expected that no problems will exist in this area since the flow of work is on a regular basis and no longer represents an interference with data processing needs.

Data retrieval on EAM (non-computer punch card equipment) has decreased substantially during the year. Most all of the demands for data have been met by the contract facilities or by the Section's programmers using the IBM 1401 at a contract facility or the Computation Branch machines. The data used was drawn from the computer data files rather than the punch card files. This process has been greatly facilitated by the acquisition of the H-200 at CDPB with the ability to convert from IBM width tapes to Honeywell width tapes. The H-200 has also expedited processing of Flexowriter output to the H-800. This has been made possible by the ability of the H-200 to translate data from punched paper tape to magnetic tape without producing punch cards as an intermediate stage.

The major use for EAM processing now is the production of punch cards containing new study data for the data files. At present, over 300,000 additional punch cards are awaiting input to the data system, probably after July 1, 1965. About 150,000 additional cards should be ready at that time.

II. Problems

The single biggest problem faced by SSDP is the lack of assurance of continuity in the data processing area. Since the Computation Branch will be unable for a number of years (at least 2 years and perhaps even longer) to provide a major aid to the SSDP in handling data processing problems, it is necessary to obtain the needed data processing facilities via contracting and other sources. At present, the longest time for which it is possible to expect continuity is 12 months. This makes it difficult to implement the long-range planning of data processing needs required in a study of this magnitude. Plans

have to be made on an alternative basis, continually taking into account inability to continue contracting with a facility in the future. Therefore, programs and other systems have to be designed on a short-range rather than a long-range basis since it is uncertain as to whether or not the additional cost of developing the long-range system will be warranted. In addition to this, certain programming techniques that are unique within a given organization must be avoided or used selectively so that the programs and other materials developed, which are the property of PRB, can be used by other services. If these programming techniques could be used freely, there would be savings both in time and money.

Because of budgetory limitations, no effort will be made to microfilm study records until after fiscal 1966. The storage of records has become a serious problem of the Section since we are running out of physical space to house the more than 2 million Study forms presently received. There are 15 thousand completed processed Study records available for microfilming. Therefore, we are faced with the need to increase the available storage space within the working quarters of the Branch. An expedient in shifting the filing of certain records from files to open shelves can be expected to ameliorate the situation for the next 12-15 months.

III. Proposed Future Objectives

In the next year it is expected that approximately 1/2 million to 3/4 million additional punch cards will be added to the data file and the data systems will be maintained and expanded. There will be a continual flow of studies produced by the Collaborative Project. The beginning of the progress in data processing, started in this current fiscal year, will expand and show a maturity and depth heretofore not possible.

In the next fiscal year, if at all possible, all punch card data files will be discarded and all data will be obtained from the tape files. Punch cards will be used for update and maintenance of the tape files only.

It is possible that by the end of the next year, and more probably in the year following, the Section will acquire a small computer facility, satellite to the large CDPB facility. It can be expected that this machine will relieve to some extent the need for contractual services. However, its size and limited scope will only handle the data file area and it will not provide adequate facilities for the large studies needed by the Branch. In fact, even after the computer is installed, it may take as long as 6-8 months before the Section will be ready to assume the full data file load. Probably extensive reprogramming will be required and for at least several months parallel processing of data and tests will be needed to insure the adequate transferability of the data from contract machine facilities to Section operated facilities. This, of course, presumes that the problems of space, personnel position, and hiring and training of personnel have all been solved on schedule.

H. SECTION ON PROJECT SERVICES
I. Medical Literature
(July 1, 1964 through June 30, 1965)

I. ACHIEVEMENTS

A. Services - General

1. Journal Reviews - a selected listing of current journal literature distributed to PRB Staff and NINDB Director's Office and from which over 15,000 reprints were requested by staff members. Approximately 150 issues of Journal Reviews are distributed over a 12-month period, averaging 3 issues a week, but in practice the number of issues a week will depend on the volume of journals received during a given week.

2. Monthly listing of "References on Congenital Malformations" compiled from current monthly issues of INDEX MEDICUS.

3. The monthly listing of "Notices of Research" (summaries of current ongoing research of particular interest to the PRB Staff) has been discontinued. This was necessitated because of a substantial increase in demand for other Services and an insufficient number of clerical assistants which would permit the fulfillment of increased demand for other services and the continuation of this listing.

4. "Demand" Service providing quick acquisition of needed literature from the NIH Library (literature not available at PRB Reading Room) and special reference searching at the National Library of Medicine. This task is assigned to one of the library assistants so that much time that might otherwise be spent by the professional staff in searching the literature stacks and commuting to NIH is spared. Over the period of this report more than 1414 books and journals have been obtained from the NIH Library and brought to PRB for staff use. The recent restriction by the NIH Library on the borrowing of single subscription journals has not decreased our effort in this regard. It has rather the effect of increasing our work because if the library assistant finds that a second loan copy is not available, she must then prepare a photocopy request and wait for the photocopy to be prepared if the particular item is needed on the same day. Time usage for this service averages 1 1/2 hours daily but a trip is made only when there are a sufficient number of requests to warrant it or if the request is urgently needed on the same day.

5. Centralized System for Requesting NIH Photocopy Service - Ever since the establishment of the coupon system by the NIH Library, all PRB staff requests for NIH Photocopy Service are processed through this Unit. This phase of the operation continues to work well and service from the NIH Library has been excellent. MLS staff members have maintained an exceptionally high level of working rapport with the NIH Library staff. This cooperative working relationship with the NIH Library has accrued to our advantage

especially in special situations and/or problems which either require "rush" handling or which require a continuing special procedure in order to resolve unusual but repetitive conditions. During this fiscal period approximately 2400 photocopy requests have been processed through this Unit, including those items selected for the PRB Reprint (Indexed) File.

6. Reading Room Services - The PRB Reading Room now contains (a) a collection of 430 medical, scientific and statistical textbooks, including anatomical atlases, scientific directories, international classification manuals, etc., all classified according to the National Library of Medicine Classification System; (b) single subscriptions to 75 journals (including 4 abstracting services) in those disciplines of critical interest to the PRB staff; (c) a locator card file giving information on a total of 440 other technical and medical books available in PRB offices other than the PRB Reading Room (135 new acquisitions processed this fiscal year); (d) a bulletin board exhibiting current medical news of interest to the Branch; (e) a reference and inventory card file on all acquisitions assigned to the PRB Reading Room.

B. Services - Special

1. Selected reference searches (requested by individual PRB staff members) from which either reference lists were prepared or selected reprints were obtained: [These services were provided by the PRB Reading Room] (a) Battered Child Syndrome [16 refs.]; (b) Smoking and Its Effects on Pregnancy [18 refs.]; (c) Cerebrovascular Diseases [Prepared by NIH Library] [26 refs.]; (d) Smoking and Cardiovascular Diseases [Prepared by NIH Library] [22 refs.]; (e) Respiration and thermoregulation in the newborn and older infant. [17 refs.]; (f) Light and Its Effect on the Diagnosis of Jaundice in Newborns; (g) Study of psychological and socio-economic factors in slum areas (23 refs.]; (h) Chromosomes and Pathological Conditions in the Stillborn [32 refs.]; (i) Newborn Infants with Birthweight of Over 4500 grams [24 refs.]; (j) Pseudo-pregnancy in Humans and Animals [26 refs.]; (k) Myopia - Etiology and Complications (Humans and Animals); (l) Sjogren's Syndrome; (m) Phenylketonuria [Prepared by the Children's Bureau]; (n) Galactosemia [Prepared by the Children's Bureau]; (o) Blood and the Rh factor [Selections from a listing prepared by the NIH Library].

2. Selected reference searches prepared for staff members of PRB Chief's Office: (a) Bacteriuria in Pregnancy and Related Subjects [Five-year survey, approximately 300 refs.]; (b) Fifteen-year survey of research publications by L. Lasagna [65 refs. and reprints processed]; (c) Current and updated listing of papers published since June 1963 and supported by Collaborative and Field Research, NINDB [Approximately 40 refs.]; *(d) Behavior Problems in Premature Children [Five-year survey;43 refs.]; *(e) Characteristics of Pre-School Children Affecting Ability to Learn to Read and Write [1963-1964 Coverage; 79 refs.]; (f) Breech Delivery, Breech Presentation and Related Problems [Three-year survey; 87 refs.]; (g) Maternal epilepsy and fetal sequelae [In process].

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*For these listings most of the articles were reviewed before being selected for inclusion.

3. Continuing reference searches from prior period: (a) Congenital malformations [drug etiology, etc.]; (b) Polyarteritis nodosa in infancy [Including any lesions involving the aorta in infancy]; (c) Hyaline membrane disease and respiratory distress syndrome; (d) Apgar scores; (e) Prenatal care and prenatal influences; (f) Single umbilical artery.

4. Working with NIH translators on special foreign-language articles occasionally requested by members of PRB staff.

C. Other Current Activities

1. PRB Central Reprint Collection and Retrieval System. The MLS biologist (and documentalist) has now indexed over 2,000 reprints and has prepared a Dictionary of Terms for documenting selected reprints according to the Termatrex System. A 3 X 5 cross reference author card file is also simultaneously prepared. In addition, there are approximately 2,000 reprints, in various stages of preparation, which will augment the present collection. Work is proceeding in the selection of the useful current literature to update the collection.

2. Bibliographies - Work continues on three long-range reference listings:

(a) Thyroid function and its relationship to fertility, pregnancy, pregnancy outcome (e.g., prematurity and mental retardation), and neonatal, mental and physical development [806 refs.].

(b) References on Prematurity (supplement to the listing published in 1961) [1915 refs.].

(c) Updating a preliminary listing of references representing clinical and laboratory research financially supported by the Collaborative Project on Cerebral Palsy, etc., NINDB.

3. Literature Support to the Information Officer Detailed to PRB. MLS alerts the Information Officer concerning current literature of significant interest to the Collaborative Study, either upon his request for information on specific topics or through various MLS literature media. MLS may also suggest items of medical news having appeal to those who read the Collaborative Project Reporter, and assists the Information Officer in the collection of published papers and/or specific medical information to satisfy press needs.

II. Problems In the Operation of Medical Literature Services

The two major problems continue to be those of:

A. Inadequate physical space for operational activities and lack of potential open storage capacity for the expected volume increase in current journal literature and updated scientific books.

B. Inadequate staffing in respect to capability in the dissemination of medical information available to MLS, and in respect to the active operation of the PRB Central Reprint File. MLS continues to maintain its operation with a personnel allotment of five full-time permanent positions and two part-time temporary appointments. Two professionals (medical officer and biologist), two semi-professionals (library assistants) and an average of two full-time clerk-typists comprise the operating staff. Woman-hours for this 12-month period are estimated as follows: Professional - 4000; Library Assistants - 4000; Clerical - 4300 (includes summer appointees).

III. Future Activities

While in the past Medical Literature Services has worked closely with individual PRB staff members and concentrated its efforts to fulfill the individual needs and assignments of a particular staff member, it is now recognized by the Chief's Office and other PRB staff members that activity in the area of literature services must in the immediate future begin to focus more and more along the lines of overall study proposals made either by the Branch or the various scientific investigators of the Collaborative Project on Cerebral Palsy, etc. Meetings with PRB Section Heads are now in process to implement a redefinition of goals for this Unit. By focusing more attention to giving literature support in those subject areas related to study proposals, this Unit will be able to assist the Branch in determining the research and publication worthiness of given proposals.

Other goals would be to give direct literature support to studies in progress or studies being prepared for publication. While various types of individual services may have to be eliminated to accomplish these new goals, strong literature support in these new areas will enhance the effectiveness of the overall service this Unit gives to the Perinatal Research Branch. The present status of PRB data processing and study proposals impels these new goals since it is fundamental and essential to all productive research to have substantial knowledge of the work of other scientists who are investigating problems in related areas.

In addition, the preparation of literature references to be fed into the computer along with certain types of study data has also been suggested. This would give staff members an immediate source of some of the literature available in certain study areas at the very beginning of the data analysis.

H. SECTION ON PROJECT SERVICES
(11) Information Service
July 1, 1964 through June 30, 1965

1. Achievements

As the Collaborative Perinatal Research Project continues to mature, new publications provide a growing resource of information for various audiences. An information specialist and editorial assistant have been assigned by the Institute Information Officer to the Perinatal Research Branch to help in maintaining a flow of information, especially for paramedical and general reporting purposes.

The Collaborative Project Reporter

The 8- to 12-page Collaborative Project Reporter is issued three times each year to Study staff (1600 copies). Its essential purpose is to help management in its continual task of lending cohesiveness, a sense of unity of mission, to a multi-institutional, multi-disciplinary study. One issue each year carries, among numerous items for a general audience, a reprint of the annual report to Congress outlining Study progress.

General Distribution of Information Materials

During the past year about 10,000 pamphlets and reports, including Institute-prepared folders on cerebral palsy, hearing loss, and other perinatal topics, were mailed from the PRB Information Service. The Information Service prepared about 420 "information sets" for orientation of new staff members and others. Each set consisted of basic pamphlets giving an overall view of Project and Institute objectives. Sets of protocols of Study forms and manuals also were distributed from time to time to professional recipients.

Public and Press Inquiries

Hundreds of public and press inquiries were handled during the year. For example, the September 1964 Readers Digest carried a Project-centered article based on information supplied by PRB. Press releases on Project findings relative to Apgar scores as forecasters of neonatal morbidity and on rubella research were widely published by newspapers and broadcast by radio and television.

Visual Aids

Numerous requests for motion picture films, professional slides, and photographs were filled during the year. In a recent two-month period approximately 100 slides of Project data were obtained through Information Service liaison with NIH art and photography groups.

Special Reports

A broader communication of information is encouraged by adapting technical materials to special reports for the NIH Director, other Service or Department officials, and the Congress.

I. SECTION ON STATISTICAL ANALYSIS
Report for Period July 1, 1964 through June 30, 1965

I. Summary of Scientific or Professional Accomplishments

Within the past year, the Section on Statistical Analysis has performed its various functions in connection with studies of placenta previa, abruptio placentae, prolapsed cord, spontaneous rupture of membranes, neonatal pneumonia, bilirubin levels, toxemia, effects of duration of labor, incompetent cervix, uterine dysfunction, maternal and fetal dystocia, age of gravida in relation to complications of pregnancy, placental weight, multiple pregnancy, organic heart disease in pregnancy, hyper- and hypothyroidism in pregnancy, coagulation defect, fetal compromise during elective caesarean section, obstetric influences on the first year development of the infant, abortion, the blood types of the gravida and her child, leiomyomas and other gynecological tumors, the associations between early registration in a maternity clinic and the outcome of pregnancy, and the history of prior pregnancies as a predictor of outcome of current pregnancy.

II. Problems

At the present time there is an urgent need for personnel who can abstract material from selected case records. Analysis of some studies requires substantially more detailed information than is available in the coded records. These personnel may be recruited from the nursing profession or from paramedical disciplines with comparable background, but with sufficient facility or acquaintance with medical terminology.

III. Role of the Section

The Section on Statistical Analysis, Office of the Chief, PRB, was organized in 1964, as data analysis, in contrast to data collection, began to become a dominant function of the Study. The role of the new Section is to offer consultative services to members of the collaborating institutions and of the Perinatal Research Branch in the design of studies, to help in the preparation of layouts for submission to the Section on Systems Design and Procedures as a preliminary to computer tabulations and computations, to abstract data from case records, and to perform appropriate tabulations and make necessary statistical analysis of data compiled by hand or machine. In addition, it designs, on its own, specific studies with the help of other professionals and engages independently in devising and applying mathematical principles and theories for mass data handling, as follows:

(1) Employment of discriminant analysis as a method of distinguishing between two mutually exclusive groups, including investigations

of various characteristics of the discriminant function and of its behavior under various conditions found in the Collaborative Project:

(a) Its stability from sample to sample

(b) Its behavior when some of the independent variables are non-normal, particularly dichotomies of scaled measurements.

(c) The effect on the function of various methods of handling cases for which there are unknowns, such as including only cases for which all items are known, estimating unknown parameters with the mean of the known cases, or estimating unknowns by a regression technique.

(2) Analyses of the relationships among certain important characteristics, for interaction effects on various outcomes, along with investigation into the effects of various transformations and multiple-variable codes on discriminant functions and regression analyses.

(3) Investigation into parallel-line regression techniques, both linear and non-linear, particularly with non-orthogonal data, with examination of the effects of various transformations and methods of weighting.

(4) Investigations into methods of analyzing categorical data, particularly where numbers of cases differ widely; techniques include multi-dimensional chi-square and analyses of variance where data are percentages.

III. APPENDIX A

STUDY PAPERS REVIEWED BY THE PUBLICATION REVIEW BOARD
For the Period July 1, 1964 Through June 30, 1965

No.	TITLE	AUTHOR
1.	Obstetrical Factors and Selected Pregnancy Outcomes	H. Berendes
2.	The Evaluation of Therapeutic Procedures in the Practice of Obstetrics	H. Berendes
3.	Obstetrical Complications and Mental Deficiency	H. Berendes
4.	Evaluation of the Problem of Breech Delivery	H. Berendes
5.	Obstetric Influences on the First Year's Development of the Premature Infant	E. Bishop S.L. Israel C. Briscoe
6.	The Relationship of Selected Characteristics of the Newborn to Neurologic Morbidity During First Year of Life	S. Drage K. Nelson H. Berendes
7.	Significance of Single Umbilical Artery	L. Froehlich T. Fujikura
8.	The Influence of Race and Other Factors on Pulmonary Hyaline Membranes	L. Froehlich T. Fujikura
9.	The H-1 and the RV Viruses and Pregnancy: Serological Study of Certain Groups of Pregnant Women	G. Monif J. Sever Wm. Cochran
10.	Fetal Morbidity Following Potentially Anoxigenic Obstetric Conditions: I. Abruptio Placentae	K. Niswander E. Friedman B. Hoover H. Pietrowski M. Westphal
11.	Fetal Morbidity Following Potentially Anoxigenic Obstetric Conditions: II. Placentae Previa	K. Niswander E. Friedman D. Hoover H. Pietrowski M. Westphal

APPENDIX A (Continued)

NO.	TITLE	AUTHOR
12.	Fetal Morbidity Following Potentially Anoxigenic Obstetric Conditions: III. Prolapse of the Umbilical Cord	K. Niswander E. Friedman D. Hoover H. Pietrowski M. Westphal
13.	The Birthweight - Placental Weight Relationship: A Statistical Analysis	A. Sedlis D. F. Stone H. Berendes W. Weiss J. Deutschberger E. Jackson H. Kim
14.	1964 Rubella Epidemic: Effect of Pregnant Women in the Collaborative Perinatal Research Study	J. Sever K. Nelson
15.	The Umbilical Cord Complications of True Knots, Nuchal Coils and Cords Around the Body	W.N. Spellacy H. Gravem R. O. Fisch
16.	Spinal Cord and Brain Stem Injury at Birth	A. Towbin
17.	Fetal Compromise During Elective Caesarean Section	R. Benson F. Shubek W. Clark H. Berendes W. Weiss J. Deutschberger
18.	Prognostic Significance at One Year of Age of Early Signs of Neurological Abnormality for Premature Infants with Birthweights of 1000-2000 Grams	S. Drage K. Nelson

APPENDIX B

STUDY PROPOSALS SUBMITTED TO PRB, NINDB
For Period - July 1, 1964 through June 30, 1965

1.	Placenta Previa	-	Friedman, Niswander
2.	Abruptio Placenta	-	Friedman, Niswander
3.	Prolapsed Cord	-	Friedman, Niswander
4.	Spontaneous Rupture	-	Shubeck
5.	Pneumonia	-	Korones
6.	Bilirubin	-	Boggs, Hardy
7.	Abortions	-	Sappenfield, Mule
8.	Cervical Dilatation	-	Friedman
9.	Placental Weight	-	Sedlis
10.	Apgar	-	Kennedy, Drage
11.	Dermatoglyphics	-	Myrianthopoulos
12.	Early Signs	-	Drage, Nelson
13.	Cord Complications	-	Spellacy, Gravem, Fisch
14.	S. U. A.	-	Fujikura
15.	Hyaline Membrane Disease	-	Drorbaugh, Cochran, Gillespie, Driscoll, Fujikura
16.	Congenital Heart Disease	-	Mitchell, Bajda
17.	Cross Tabulations for Transformations		
18.	Iterative Procedure for Discriminant Analysis		
19.	Blood Pressure	-	Mule, Sappenfield
20.	Premature Rupture of Membranes	-	Sappenfield, Mule
21.	Prematurity	-	Bishop, Westphal

22.	Mental and Motor Retardation	-	Lassman, Westphal, Fisch, Turoff
23.	Placental Insufficiency		
24.	Anesthesia		
25.	Resuscitation		
26.	KUB		
27.	Height, Weight	-	Milan, Preston, Shakhashiri
28.	Uterine Dysfunction and Dystocia	-	Gates, Vosburgh
29.	Staining		
30.	Pregnancy Care	-	Hardy
31.	Twins	-	Brooks, et al
32.	Prior Pregnancy Loss	-	Brooks, et al
33.	Duration of Labor	-	Niswander, W.A. Reid, Bishop
34.	Incompetent Cervix	-	Gillespie
35.	Toxoplasmosis	-	Sever, Hardy, Milan, Drage
36.	Toxemia	-	Niswander, Mule, Sappenfield, Sever, Westphal
37.	Twins	-	Tretter
38.	Blood Types and Outcome	-	Bishop et al
39.	Spontaneous Abortions	-	Naylor
40.	Age of Gravida	-	Israel
41.	Organ Weights Study	-	Lipkin
42.	Race and Complications of Pregnancy	-	Korones, Patterson
43.	Effect Maternal Hyper- and Hypo-thyroidism Outcome Pregnancy	-	Hardy

44.	Evaluate Neurological Status of Children born Following Electively Induced Labor	-	Low, Gates, Carter
45.	Glomerulonephritis In Gravida on Outcome of Pregnancy	-	Berendes
46.	Women in Collaborative Project With The Diagnosis of Coagulation Defect	-	Abailli
47.	Congenital Anomalies, Tumor Lower Brain Stem, Retinal Hemorrhage	-	Leventhal
48.	Hydramnios	-	Gorbach
49.	Neonatal Pneumonia	-	Korones
50.	Effects of Cardiovascular Disorders in Preg. Women on Offspring as Described by 8-Month Psych. Exam.	-	Mendelson, et al
51.	Groupings of Indices on the Neonatal Exam. of PED-2 and Their Relationship to Mental and Motor Scores on the 8-Month Psych. Exam.	-	Mendelson, et al
52.	Public Health Implications	-	Shakhashiri, et al

APPENDIX C

ROUTINE FEEDBACK ON POOLED DATA
For Period July 1, 1964 through June 30, 1965

Series IX - (tables 1-13) - Mailed 8/21/64

PED-7 Nursery Summary by Study Institution

Series X - (tables 1-21) - Mailed 8/21/64

PED-8 Nursery Summary by Study Institution

Series XI - (tables 1 & 2) - Mailed 8/21/64

Frequency Distribution by Hospital of OB-60 Disease and Condition Codes (Total History and During Pregnancy) by Study Institution

FREQUENCY DISTRIBUTIONS

Book I - Mailed 2/12/65

AR-1 Obstetrical Administrative Record
OB-9 Prenatal Record
OB-10 Laboratory Data

Book II - Mailed 2/19/65

OB-5 Recent Medical History
OB-7 Infectious Disease & Systems Review
OB-34 Cesarean Section Data
OB-37 Labor Data
OB-44 Prenatal Observations
OB-57 Anesthetic Agents

Book III - Mailed 2/26/65

OB-60 Obstetric Diagnostic Summary
(conditions during pregnancy vs. BIRTHWEIGHT)

Book IV - Mailed 3/5/65

OB-2 Reproductive History
OB-5 Recent Medical History
OB-34 Obstetrician's Summary of Labor & Delivery

Book V - Mailed 3/12/65

OB-60 Obstetric Diagnostic Summary
(conditions during pregnancy and other times vs. OUTCOME)

Book VI - Mailed 3/19/65

OB-6 Past Medical History
OB-9 Prenatal Record

Book VII - Mailed 3/26/65

OB-60 Obstetric Diagnostic Summary
(conditions during pregnancy and other times vs. CONGENITAL MALFORMATIONS as reported on PED-8)

Book VIII - Mailed 4/2/65

PS-1 Eight-Month Psychological Examination

Book IX - Mailed 4/9/65

OB-60 Obstetric Diagnostic Summary
(SUMMARY TABLES and conditions during pregnancy vs. ONE MINUTE APGAR, FIVE MINUTE APGAR, NEUROLOGICAL ABNORMALITY)

Book X - Mailed 4/16/65

PED-8 Newborn Diagnostic Summary

Book XI - Mailed 4/23/65

PED-2 Neonatal Examination

Book XII - Mailed 4/30/65

OB-60 Obstetric Diagnostic Summary
(conditions during pregnancy vs. MENTAL SCORE)

Book XIII - Mailed 5/7/65

OB-60 Obstetric Diagnostic Summary (conditions during pregnancy vs. MOTOR SCORE)

Book XIV - Mailed 7/14/65

FHH-1,3 Family Health & History
GEN 5-8 Family History Interview
ADM-44 Report of Non-Liveborn Termination or Death of Study Child

APPENDIX D

PARTICIPATION IN SCIENTIFIC SESSIONS, PERINATAL RESEARCH BRANCH, NINDB
For Period July 1, 1964 through June 30, 1965

1. Meeting on Infant and Perinatal Mortality, NICHD, Bethesda, Maryland, July 8, 1964.

2. International Copenhagen Congress on the Scientific Study of Mental Retardation, Copenhagen, Denmark, August 7-14, 1964. Presented paper, "Obstetrical Complications and Mental Deficiency."

3. IVth World Congress of International Federation of Gynecology and Obstetrics, Buenos Aires, Argentina, September, 1964. Presented paper on "The Evaluation of Therapeutic Procedures in the Practice of Obstetrics."

4. Symposium on Effects of Labor on the Fetus and Newborn, Montevideo, Uruguay, October, 1964. Presented paper on "Evaluation of the Problem of Breech Delivery."

5. Participated in Conference on Diabetes in Pregnancy, Charter House, Cambridge, Massachusetts, October 18, 1964.

6. American Academy of Cerebral Palsy, New York City, December 11, 1964, panel presentation on Status of the Collaborative Project.

7. Meeting of working group on Manual on Congenital Malformations, January 18-20, 1965, Pan American Health Organization, WHO, Washington, D. C.

8. Colloquium on Longitudinal Studies, February 7-10, 1965, Old Point Comfort, Virginia. Discussions on "Problems of Human Development."

9. American College of Obstetrics and Gynecology, San Francisco, California, April 3-7, 1965. Seminar on Perinatal Mortality and Morbidity. Made presentation on "Findings of the Collaborative Study of Cerebral Palsy in Regards to Perinatal Mortality and Morbidity.

10. 4th Annual Conference on the Premature at Stanford University Medical School, May 14, 1965, Palo Alto, California.

11. Congress of the Illinois Association of Maternal and Infant Health, Springfield, Illinois, February 18, 1965, presented paper on "Indicators of Morbidity in the Newborn."

12. Society for Research in Child Development, Minneapolis, Minnesota, March 26, 1965, presented paper on "The Relationship of Selected Characteristics in the Newborn to Neurological Morbidity During the First Year of Life."

13. American Pediatric Society, Philadelphia, Pennsylvania, May 5, 1965, presented paper on "1964 Rubella Epidemic: Effect on Pregnant Women in the Collaborative Perinatal Research Study, Bethesda, Maryland."

14. Society for Pediatric Research, Philadelphia, Pennsylvania, May 6, 1965, presented paper on "Toxoplasmosis: Serological Clinical Studies of 23,000 Pregnant Women. A Report from the Collaborative Perinatal Research Study."

Serial No. NDB(CF)-57 PR/ID 402
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

PART A:

Project Title: Sero-Epidemiology of Infectious Diseases in the Collaborative Study on Cerebral Palsy, Mental Retardation, and other Neurological and Sensory Disorders of Infancy and Childhood.

Principal Investigators: Dr. John L. Sever, Dr. Akinyele Fabiyi, Dr. Robert J. Huebner

Other Investigators: Dr. Gilles Monif
Dr. Charles Cusumano
Dr. Richard Hildebrandt
Anita Ley
Flora Wolman
Renee Traub
Mary Ruth Gilkeson
Collaborative Institutions in the Perinatal Research Study

Cooperating Units: NIAID, Laboratory of Infectious Diseases (Dr. Robert J. Huebner)

MBA - Dr. Gabriel Castellano

Cooperating Institutions in California and Hawaii (with the Section on Infectious Diseases)

Man Years:

Total:	9-12
Professional:	9-12
Other:	0

Project Description:

Objectives: To utilize new serologic techniques in an intensive study of viruses, protozoa, and bacteria, as to their relation to birth defects and related abnormalities. To develop, wherever technically possible, the serologic methods necessary for a large scale investigation of the natural course of the disease as caused by infectious agents.

Methods Employed: Serologic techniques, including complement fixation tests, hemagglutination-inhibition, hemadsorption, viral neutralization and tissue culture neutralization tests are now available for the identification of over 120 viral, protozoal, and bacterial infections.

The first phase of this project is the production and standardization of antigens and antisera for the performance of the serological tests. The viral agents produced under the research and development contract with Microbiological Associates, Inc., in consultation with the Virology Sections, LID and NINDB, together with the direct purchase of viral reagents constitutes the materials utilized for the serological investigations. These reagents are essential for the continuation of the studies.

The second phase of this project is the development and utilization of an integrated laboratory facility, employing and training technicians capable of handling large scale testing and development of new serological methods.

The third phase of the project is to utilize the extensive serological data available from the tests in conjunction with the clinical information available from the Perinatal Research Study to determine the possible relation between the infecting agents and the outcome of the pregnancy or abnormalities of the individuals.

Major Findings: A total of 100 reactive antigens have been developed. Approximately two-thirds of the antigens have been thoroughly evaluated and are now being applied in routine testing of study sera. The development and maintenance of large quantities (1,000 ml) of satisfactory antigens for 40 viruses is an integral part of the investigation being carried on by the study. The other antigens are receiving intensive developmental work and 40 of these antigens are under test for specificity. Specific control antisera have been prepared from 75 virus serotypes. In addition, to provide improved safety, extensive work has been conducted on the inactivation of the live virus antigens.

The serological studies are conducted in accordance with three major study designs:

First, epidemiological studies to determine the frequency of virus experience among study populations. Specimens from representative patients at study hospitals are tested for evidence of antibody. By testing these specimens, it is possible to establish the frequency of antibody and change in antibody titer to each virus. The data for each hospital is then analyzed in relation to other information from the Collaborative Perinatal Study, and epidemiological data concerning the occurrence of abnormal pregnancies and children.

The second category of study design involves the selection of special pregnancy outcomes for intensive testing. Studies of this type have involved, for example, the testing of sera from all study patients for antibody to toxoplasmosis and special investigations of rubella. Other studies have included specific patients who have had unusual obstetrical histories or defective children for whom special serological testing is indicated.

Third, studies are designed to obtain maximum data concerning the virus experience of patients with abnormal pregnancy outcomes, and patients who are "matched controls". The results of this type of study can then be analyzed in terms of differences in frequency of antibody among the abnormals and matched controls. The matching of the patients include factors which are known to influence virus experience, such as time of the year during which the specimen was obtained, race, age, number of living children in the family, and geographic location of the patients. These studies are conducted when a sufficient number of abnormals of a particular type have been identified so that statistical analysis might establish valid information. The initial studies were directed at abnormalities which are relatively frequent, such as abortions, stillbirths, and neonatal deaths. The less frequent abnormalities or those which cannot be recognized in infancy or early childhood are being studied as greater numbers of these patients are identified in the Collaborative Study population.

The data from the three types of study design outlined above is analyzed with the assistance of statisticians in the Section, Branch, Institute, and Institutes. All data is analyzed daily for the reproducibility of the testing itself. A minimum standard of 90% reproducibility is required for all testing.

Studies to date have demonstrated information concerning the extent of virus experience in the study population. These investigations indicated at least 22% of the pregnant study population tested had evidence of exposure to one or more of the 100 viruses which have been studied. The effect of the 1964 epidemic of rubella on the Collaborative Study Population is being investigated. Multiple specimens are being tested to identify patients with inapparent rubella and to document clinical rubella. These studies are directed towards investigations of prevalence of virus experience in the population under surveillance. In addition, specific studies of infection among patients whose pregnancy terminated in an abnormal outcome are also being conducted. Studies are being directed toward abnormal pregnancy outcomes which can be recognized shortly after birth, such as abortions, still births, and neonatal deaths and congenital malformations. Several viruses have been found to occur in high frequency in specific patients with abnormal pregnancy outcomes.

Serum specimens from pregnant women participating in the Collaborative Perinatal Research Study at 12 hospitals located throughout the United States were tested for antibody to toxoplasmosis. The tests employed a micro modification of the hemagglutination technique of Jacobs and Lunde. Of the patients studied, 38.4% demonstrated titers of 32 or greater, 11.7% had titers of 256 or greater, and 2.2% had titers in the range considered to be "suspect" for recent or active toxoplasmosis. Significant increases in antibody during pregnancy were identified for approximately 2% of the patients and for one tenth of this group, there was evidence for primary exposure. The greatest frequency of antibody was found in specimens from patients in New York City. Antibody titers were correlated with the age of the patients.

New studies are being conducted on the association between multiple sclerosis and antibody to measles virus. In addition several other viruses were tested in the clarification of the role, if any, in the etiology of multiple sclerosis. Complement fixation, hemagglutination inhibition and neutralization tests were conducted with the cerebraspinal fluid and sera of multiple sclerosis patients and controls.

Significance to the Program of the Institute: The use of the micro serological techniques for a large group of new viruses provides an opportunity to investigate the course of human disease caused by viruses which are either difficult to isolate or are resistant to evaluation because the clinical effects are delayed until a long time after infection has subsided. This is particularly true in the case of birth defects. The application of this tool for analysis is providing valuable information on the epidemiological aspects of virus infections.

Proposed Course of the Project: The serological program will be expanded in terms of antigenic materials and the collection of sera. New study arrangements for investigation of viral and genetic causes of abortions are being developed.

As additional abnormal pregnancy outcomes are reported, these will be added to existing studies on abortions, stillbirths, neonatal deaths, and congenital malformations.

The initial "leads" obtained by the present serological testing will be explored in detail with the use of expanded serological investigations as well as other techniques of virology and by new study designs involving the intensive sampling of particular groups. It is already clear that supplemental sampling will be necessary for patients who register early in the first trimester of pregnancy and for those who have had repeated abnormal pregnancies. Arrangements are being developed for obtaining full sets of serum specimens and data for patients who abort or have stillbirths. Finally, it will be most important to extend specimen collection for virus studies as soon as possible to include tissue samples from placentas, abortuses, stillbirths, and neonatal deaths so that direct isolation of viral agents may be included to confirm and extend the serological findings.

The existing research and development of virus diagnostic reagents will continue in the following six main areas: (1) The development of 20 virus antigens and testing of specificity; (2) the development of 60 specific control antisera for use in the serological studies; (3) production of large quantities (1,000 ml) of CF antigen for rubella; (4) the development of improved methods of storage of antigens and antisera prepared under this contract; (5) the preparation of specific antigens which require special developmental work each time they are prepared because of variation in the biological systems used; and (6) the preparation of histological sections of tissues prepared by the Section on Virology.

Other studies will be conducted in cooperation with the Epidemiology Branch, NINDB. More intensive serological studies will be initiated on the role of viruses in the etiology of infectious mononucleosis.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Sever, J. L., Castellano, G. A., Pelon, W., Huebner, R. J., and Wolman, F.: Inactivation of the Infectivity of Viral Hemagglutinating Antigens with the Use of Betaprone, Journal of Laboratory and Clinical Medicine, 64: 983-988, December, 1964.

Brody, J. A., Sever, J. L., McAlister, R., Schiff, G. M., and Cutting, R.: Rubella Epidemic on St. Paul Island in the Pribilofs, 1963. I. Epidemiologic, Clinical, and Serologic Findings, J. A. M. A. 191: 619-623, February 22, 1965.

Sever, J. L., Brody, J. A., Schiff, G. M., McAlister, R., and Cutting, R.: Rubella Epidemic on St. Paul Island in the Pribilofs, 1963. II. Clinical and Laboratory Findings for the Intensive Study Population, J. A. M. A., 191: 624-626, February 22, 1965.

Sever, J. L., Schiff, G. M., Bell, J. A., Kapikian, A. Z., Huebner, R. J., and Traub, R. G.: Rubella: Frequency of Antibody Among Children and Adults, Pediatrics, In Press.

Sever, J. L., Huebner, R. J., Castellano, G. A., Sarma, P., Fabiyi, A., Schiff, G. M., Cusumano, C. L.: Rubella Complement Fixation Test, Science, 148: 385-387, April 16, 1965.

Serial No. NDB(CF)-61 PR/ID 835
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Clinical Investigations in Human Volunteers and other Populations of Virus Effects and Production of Prototype Human Antisera and Vaccines

Principal Investigators: Dr. John L. Sever, Dr. Charles Cusumano, Dr. Robert J. Huebner

Other Investigators: Dr. Richard J. Hildebrandt
Dr. Gilles Monif

Cooperating Units: NIAID, Laboratory of Infectious Diseases
(Dr. Robert J. Huebner)

Bureau of Prisons, Department of Justice
Mr. Myrl Alexander, Director
Dr. Charles E. Smith, Medical Director
Petersburg Reformatory
Warden David Hertige and Associates

Chanute Air Force Base
United States Air Force Hospital
Chanute, Illinois
Captain John R. Baringer

Naval Medical Research Unit No. 4
Great Lakes Hospital
Great Lakes, Illinois

Dr. Paul McCallin, Honolulu, Hawaii

Man Years:

Total:	4-12
Professional:	4-12
Other:	0

Project Description:

Objectives:

A. Study the effects of specially selected viruses on human volunteers and other populations to determine the pathogenicity of these agents (as rubella), the immunity which is developed.

B. Develop prototype human antisera for controlled virus studies as in A and project (Serial No. NDB(CF)-62 PR/ID 972).

C. Study the efficacy of prophylactic and therapeutic materials for the prevention and control of infectious diseases.

Methods Employed:

Human volunteer studies are conducted in collaboration with the Federal Bureau of Prisons. These studies are reviewed and approved by the Clinical Research Committee and the Medical Board of the National Institutes of Health. Additional study arrangements were developed for studies of epidemics and vaccines.

Major Findings:

Several volunteer studies were initiated to study: 1) the efficacy of killed vaccines for rubella and 2) the value of Amantadine for the control of rubella. These studies are now being analyzed.

Investigations of virgin epidemics in Alaska provided valuable data on the spread of epidemics of rubella, the clinical findings in the disease, and the value of gamma globulin.

A joint study of rubella convalescent gamma globulin is now in progress during the epidemic of rubella in Hawaii. Combined clinical and serological data will be available.

The "Committee on Rubella Virus" has been developed to review the areas of investigations with rubella which require study to develop a satisfactory vaccine for rubella.

Significance of the Project:

Volunteer studies provide a valuable contribution to the study of infectious agents and the development of specific immune sera. The studies of epidemics have permitted the clarification of disease patterns which are important to the prevention and control of infections of significance in the perinatal period and later.

Current studies of gamma globulin should provide valuable information on the usefulness of this material. The Rubella Virus Committee is active in footing research in areas important to the development of satisfactory vaccines.

Proposed Course of the Project:

Additional studies are necessary on the value of new vaccines for the prevention of rubella. In addition, quantities of specific human sera are necessary for investigations of cytomegaloviruses. Further investigations of epidemics of agents important in the perinatal period will be conducted.

Further studies of titered gamma globulin are necessary to establish optimal conditions for the use of this material. In addition, the effectiveness of new chemotherpeutic agents is being studied. Other studies with viruses being investigated in the Collaborative Perinatal Research Program will be initiated. The recommendations of the Committee on Rubella Virus will be reviewed and implemented insofar as possible.

Part B Included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Brody, J. A., Sever, J. L., and Schiff, G. M.: Prevention of Rubella by Gamma Globulin During an Epidemic in Barrow, Alaska, in 1964, New Eng. J. Med., 272: 127-129, January 21, 1965.

Serial No. NDB(CF)-62 PR/ID 972
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Experimental Animal, Tissue Culture, Histopathological and Serological Investigations of the Role of Viruses and Other Micro-Organisms in the Perinatal Period.

Principal Investigators: Dr. John L. Sever, Dr. Richard J. Hildebrandt, Dr. Robert J. Huebner

Other Investigators: Dr. Akinyele Fabiyi
Dr. Gilles Monif
Dr. Charles L. Cusumano
Renee Traub
Flora Wolman
Bernice Caplan
Pernell Crockett
Jean Roberts

Cooperating Units: NIAID, Laboratory of Infectious Diseases
(Dr. Robert J. Huebner)

NINDB, Laboratory of Perinatal Physiology,
San Juan, Puerto Rico
(Dr. Ronald E. Myers, Dr. Gilbert W. Meier)

NINDB, PRB, Section on Pathology
(Dr. Toshio Fujikura, Dr. Lewis Lipkin)

Man Years:

Total:	10-12
Professional:	10-12
Other:	0

Project Description:

Objectives:

A. Infect Pregnant and non-pregnant animals of a number of different species with various viruses and other micro-organisms to determine the effect of these agents on the animals and the fetal tissues.

B. Attempt to recover infectious agents responsible for abnormal fetal development.

C. Correlate A and B with histopathological findings.

D. Correlate A, B and C with serological findings.

Methods Employed: An integrated investigation of the role of viruses and other microorganisms in the perinatal period by the combined use of experimental animals; tissue culture techniques; histopathological studies; and serological testing:

A. Pregnant and non-pregnant animals of various species including monkeys are being infected artificially with viruses and other microorganisms and being observed for evidence of disease and/or effect on fetal tissues. Some of these animal studies are being conducted in cooperation with the Laboratory of Perinatal Physiology.

B. Virus isolation investigations utilizing new tissue culture techniques to study the location of virus infection produced (part A) and to recover viruses from tissues of experimental animals.

C. Collaborative histopathological studies are conducted on specimens obtained in A and B.

D. Extensive serological studies are conducted with the many viral antigens developed for the collaborative study (Serial No. and new antigens with materials being studied in A, B and C.

Major Findings:

Animal investigations with 5 pregnant monkeys which had only minimal human contact demonstrated that gargle material from a patient with rubella produced fever, lymphocytosis, and leukopenia in an inoculated animal. Two animals administered control-tissue-culture fluid had no hematological changes. None of the animals developed rash or adenopathy. Neutralizing antibodies developed in both of the monkeys, Virus was recovered from the infected animals from the 6th through the 12th day. Passive transfer of antibody occurred.

The availability of this animal model system for experimental rubella would be of particular value in the development of vaccines for rubella and studies of the mechanism of pathogenesis of the disease. In addition, studies with ferrets were undertaken to determine the response of this mammal to experimental rubella infection. Tissue culture propagated rubella virus (RV) was inoculated intranasally into groups of weanling ferrets. The presence of experimental infection was documented by recovery of virus from the anterior and bony turbinates, lungs, and spleens of the animals up to 14 days after inoculation. Virus was not recovered from CNS

tissue. Significant neutralizing antibody developed after viral administration. These studies suggest that the ferret might be suitable for many types of investigations with rubella virus. Preliminary investigations with the drug Amantadine indicate this drug may be useful for suppressing infection with rubella.

Similar studies are now under way with mumps and herpes simplex. We are now extending our studies of Amantadine to include pregnant Rhesus monkeys. Isolation facilities are available for these monkeys in our laboratories. Thorough studies have demonstrated that pre-existing antibodies will not be a factor in these studies. The animals will not be more than 30 days pregnant at the time of delivery and will be determined to be pregnant by rectal palpation.

Experimental studies of H-1 and H-3 virus in hamsters showed the development of congenital malformations. These studies are being extended.

Significance to the Program of the Institute: The broad program of experimental animal, tissue culture, histopathological investigations complements the strict serological approach being used with human sera (Project Serial No. NDB(CF)-57 PR/ID 402) and presents a balanced investigation of the role of viruses and other microorganisms in the perinatal period. It provides a means of integrated direct experimental investigation of microorganisms which may contribute to perinatal pathology.

The information already gained on rubella, through these studies, opens the possibility of the development of much more information on this disease and its teratogenic effects; possible development of a vaccine; and the production of hyperimmune rubella gamma globulin.

Proposed Course of the Project:

Further studies of experimental rubella infection of ferrets and monkeys are in progress. Investigations with new vivostatic drugs are being initiated in these model systems. Vaccine studies are also being initiated in these experimental animals.

Salivary gland virus, herpes simplex, and mumps are also being studied in experimental animal models.

Part B Included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Cusumano, C. L., Schiff, G. M., Sever, J. L., and Huebner, R. J.: Rubella Virus: Nucleic Acid Studies Using 5 iodo 2'-deoxyuridine, J. Pediatrics, 64: 138-140, July, 1964.

Monif, G. R., Sever, J. L., and Cochran, W. D.: The H-1 and the RV Viruses and Pregnancy: Serological Study of Certain Groups of Pregnant Women, Pediatrics, In Press.

Serial No. NDB (CF) -65 PR/ID 1238
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Isolation of Infectious Agents from Tissues and Chromosomal Studies.

Principal Investigators: Dr. John L. Sever, Dr. Richard Hildebrandt, Dr. Charles L. Cusumano, Dr. Robert Huebner

Other Investigators: Dr. Gilles Monif
Renee Traub
Jean Roberts
Flora Wolman
Pernell Crockett

Man Years:
Total: 8-12
Professional: 8-12
Other: 0

Project Description:

Objectives:

To utilize new techniques for the isolation of infectious agents from fetal and adult tissues; and to conduct studies of the chromosomes of aborted and term fetal tissues as combined efforts to obtain direct information on the causes of abnormal pregnancy outcomes and pathologic findings.

Methods Employed:

Tissue specimens from fetuses and adults are being obtained from collaborating physicians and institutions. These specimens are being studied intensively by laboratory techniques including tissue culture and histopathology and in some cases, electron microscopy.

Major Findings:

Rubella virus was isolated from 60% of 50 fetuses which were obtained at therapeutic abortions for maternal rubella. Several patterns of recovery were noted. Virus was recovered from the tissues of 3 children who died with congenital rubella defects at 3 months of age.

Significance of the Project:

Direct isolations of agents from involved tissues provide valuable data on the probably role of the agent as the cause of the defects. Considerable new information has recently been obtained in this way for rubella.

Proposed Course of the Project:

A full time cytogeneticist will be brought into the Section for expanded studies of the genetic aspects of defective pregnancies. New studies are needed concerning the role of mumps in endocardial fibro-elastosis.

Part B included: Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Monif, G. R., Avery, G. B., Korones, S. B., and Sever, J. L.: Postmortem Isolation of Rubella Virus from Three Children with Rubella-Syndrome Defects, Lancet, April 3, 1965.

Monif, G. R., Sever, J. L., Schiff, G. M., and Traub, R. G.: Isolation of Rubella Virus from Products of Conception, Am. J. Obstet. & Gynec., April 15, 1965.

Serial Number NDB (CF)-63 PR/OC 1144
1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: An Instrument For the Conduct of A Retrospective Study of Seizures, Cerebral Palsy, Mental Retardation And Other Neurological and Sensory Disorders of Infancy and Childhood.

Principal Investigators:

Z. A. Shakhashiri, M.D.
Lenore Bajda, M.D.
Leonard V. Phelps
Blanche L. Vincent, S. N. O.
Zula C. Meekham, B. S. N.
Rose R. Tortorella

Cooperating Units:

Georgetown University Hospital, Retarded Children's Clinic, Selected Maternity Hospitals and Physicians in Metropolitan Washington.

Man Years:

	F. Y. 1965
Total:	1.92
Professional	1.32
Other	.60

Project Description:

The Project was to design and test an instrument for the conduct of a retrospective study of seizures, cerebral palsy, mental retardation and other neurological and sensory disorders of infancy and childhood. It was designed to test certain basic and important hypotheses concerning the occurrence of neurological damage. Clearly recognized damaged outcomes of pregnancy, such as seizures, diplegias, hemiplegias and choreoathetoids are to be studied and related to defined perinatal or postnatal events. The outcomes to be studied were selected because they were construed to be related to or manifestations of or involved in the biological or psycho-sociological

mechanism underlying the hypotheses. The hypotheses are eight in number as follows: (1) anoxia, (2) toxic infuences on the brain, (3) metabolic influences, (4) trauma to the head, (5) infection of the brain, (6) dehydration of the child, (7) genetic or familial patterns and (8) socio-economic status.

The pretest of the Instrument is now continuing in progress. More than 300 clinic records of mentally retarded children at Georgetown University Hospital have been medically abstracted and constitute the partial completion of data concerning the damaged child. Socio-economic information has also now been completed for the families of the damaged cases and for sib controls. Part of the socio-economic data concerning the non-sib controls has also been completed.

Part B included No

Serial Number NDB (CF)-63 PR/OC 1146
1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Revision and Expansion of Previous Project entitled A Commentary on The Appropriateness of The Use of Certain Tabular Data, For Formulating Generalizations Concerning Populations in the Same Cities As Those In Which The Collaborative Study on Cerebral Palsy, Mental Retardation and Other Neurological And Sensory Disorders of Infancy and Childhood Is Being Conducted.

Principal Investigators:

Z. A. Shakhashiri, M.D.
Leonard V. Phelps

Cooperating Units:

The Census Bureau and the National Center for Health Statistics cooperated in the furnishing of necessary statistical information for the United States and cities.

Man Years:

	F. Y. 1965
Total:	.69
Professional	.48
Other	.21

Project Description:

The purpose of the Project was to expand and revise the original project, so that the statistical information previously made available would be provided for a more extended time period and in somewhat more detail. The statistical information included both numeric tables and charts for 11 of the cities in which the Collaborative Study is being conducted for both the Collaborative Study population and the City area in which the Study hospital is located. It included the number of live births, deaths under one year and death rates per 1,000 live births; deaths under 28 days of age, 1-11 months and corresponding death rates for 1,000 live births; stillbirth-neonatal death rates

per 1,000 live births; live births of 2,500 grams or less per 1,000 live births; for Study hospital population, deaths and death rates of infants under 24 hours of age and also under 7 days of age. These data are not published for the cities. Number and percent distribution of the female population 15-44 years of age in the cities (1960 Census) and number of women registered in the Collaborative Study.

All of the above information is shown according to color, white and non-white. Some tables are included for sex and color. These data constitute a ready reference of statistical information pertaining to the infant death rates, numbers of deaths and percent distributions by color. A brief summary review of the more salient facts concerning infant mortality is provided.

Part B included No.

Serial No. NDB (CH)-65 PR/OB 1268
1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Methods and Problems in the Epidemiologic Study of Perinatal Data (Yale Report)

Principal Investigator: Rudolf F. Vollman, M.D., Perinatal Research Branch, NINDB

Other Investigators: John L. Farris
Gregory J. Ladas
Alan L. Scriggans — Medical Summer Students
Carol J. Shalek
Donald M. Sledz

Cooperating Units: None

Man Years
Total: 1.90
Professional: 1.60
Other: 0.30

Project Description:

Objectives: To utilize all obstetric cases contributed by one collaborating hospital for the design of tables that will demonstrate the relationship of major obstetric study variables.

Methods: After identification of basic variables, an abstract sheet and a manual with definitions of conditions for a standardized case summary was designed. The layout of the abstract sheet was tested and modified so that hand tabulations could easily be produced.

Major Findings: The tables show the race, age, and marital status composition in the sample. Negro women tend to register later in pregnancy than white women. By calendar month of registration, more Negro women register during the summer months than white mothers. The age of the gravida does not seem to affect the time of registration with the clinic. A very strong association between age and parity, independent of race, was demonstrated. The age at menarche does not seem to be related to pregnancy wastage. The reported length of the menstrual cycle prior

to conception has a greater variability in the white than in the Negro mother. The number of antepartum visits is clearly related to the time of registration and to the presence of significant prenatal pathology. It was a surprise to find that certain specified conditions in the past medical history (urinary disease, systemic hypertension, rheumatic heart disease, anemia, diabetes, cancer, thyroid conditions, venereal disease) show no significant racial difference in incidence rates. There are too few cases to analyze a possible association of bleeding during pregnancy with prenatal mortality or of bleeding during pregnancy with bleeding during labor. The hematocrit value drops slightly in the course of pregnancy. In the later months of pregnancy a higher percentage of low hematocrit readings was found in Negro mothers than in white women. No difference between the races in position or presentation of the fetus prior to delivery has been discovered.

Toxemia and complications during delivery could not be evaluated because of small numbers of cases. The length of the first and second stages of labor vary, of course, with the age of the mother and parity and are independent of race. Delivery was more frequently observed by Project personnel in Negro than in white mothers. The sex ratio was found to be higher in the white than in the Negro babies.

These tables serve as a descriptive inventory of the Study population in one collaborating institution. They may be used for comparative studies between hospitals and for the design of more specific investigations of associations between perinatal factors.

Part B included No

Serial No. NDB (CF)-65 PR/OB 1269
1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Fifty Years of Research on Mammalian Reproduction

Principal Investigator: Rudolf F. Vollman, M.D., Perinatal Research Branch, NINDB

Other Investigators: None

Cooperating Units: Staff Members of the NIH Library, Reference Section

Man Years
Total: 0.9
Professional: 0.1
Other: 0.8

Project Description:

Objectives: To provide references on basic data on comparative reproductive physiology.

Methods: By compilation of the bibliography of a researcher who has been deeply involved during the past 50 years in all the facets of mammalian reproductive physiology, a cross section of the major problems and a summary of the present status of this area will be presented.

Major Findings:

1. Factors affecting spermatogenesis are still largely unknown.

2. The optimal conditions for sperm migration and survival in the female genital organs are difficult to measure.

3. Several fairly precise and specific techniques for timing ovulation in experimental animals have been tested but their application in the human is still hampered by technical difficulties.

4. The biochemical conditions of the processes involved in the fertilization of the mammalian ovum are only partially known so far.

5. In all mammals a high loss rate of fertilized ova prior to implantation and in the very early stages of implantation is common. Conclusive evidence is now available that pregnancy wastage is highest in early pregnancy in women too.

6. The duration of pregnancy is a typical biological variable with a measurable spread of dispersion.

7. The length of the estrous cycle and the menstrual cycle is related to the age of the female.

8. There is an optimal time in life for reproductive processes to perform with the highest success and lowest complication rates.

9. Experimental and comparitive reproductive physiology should be more intensively used to study complications in human pregnancy and perinatal problems.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Author: Vollman, R. F.: Fifty Years of Research on Mammalian Reproduction, A Bibliography of the Scientific Publications of Carl G. Hartman. U.S. Dept. of Health, Education, and Welfare, Public Health Service Publ. No. 1281, Public Health Bibliography Series No. 57. Washington, D. C., U.S. Govt. Print. Off., 1965, 36 pp.

Honors and Awards relating to this project: None

Serial No. NDB-(CF)-63 PR/PN 1153
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: The Relationship of Birth Order to the Apgar Score of Twins.

Principal Investigators: F. E. Yazbak, M.D., Brown University
M. G. Freeman, M.D., Brown University
R. E. Holden, Ph.D., Brown University
J. Deutschberger, PRB, NINDB
Benjamin K. Schwarz, PRB, NINDB
Joseph S. Drage, M.D., PRB, NINDB

Cooperating Units: Perinatal Research Branch, NINDB

Man Years:	F.Y. 1965
Total:	1/12
Professional:	.5/12
Other:	.5/12

Project Description: There is a general medical agreement that the second born of a pair of twins is at greater risk, although there is less agreement as to the extent of the effect of birth order. The Apgar score is an index of the status of an infant one minute after its birth. The study relates the birth order of twins to their respective Apgar scores, using data supplied by the Collaborative Project for 226 sets of twins born of vaginal deliveries at the Collaborating Institutions.

The pairs of twins were assigned to four categories by sex - both males, both females, first twin male and second female, first twin female and second male. Computations of difference in total Apgar score and in the score for each of the five components of the Apgar rating were made for each pair of twins.

It is readily apparent that the Apgar score differentiates between the first and second twin; moreover, each of the individual components of the Apgar score makes a distinction. If the Apgar score is a valid indicator of the development of the child, then the findings of this

study would suggest that the heavier risk of the second twin extends beyond the fetal and neonatal periods.

Study in progress.

Part B Included: No.

Serial No. NDB-(CF)-63 PR/PN 1154
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Apgar Score - An Index of Infant Mortality.

Principal Investigators: Joseph S. Drage, M.D., PRB, NINDB
Benjamin K. Schwarz, PRB, NINDB
Charles Kennedy, M.D., Children's Hospital of Philadelphia

Cooperating Units: Children's Hospital of Philadelphia, Pennsylvania

Man Years:	F.Y. 1965
Total:	2/12
Professional:	1/12
Other:	1/12

Project Description: The Collaborative Study has included a recording of 17,229 Apgar scores one minute, and 17,534 scores five minutes after birth.

There is an observed difference in the distribution of one-minute Apgar scores by race; white infants show an increased frequency of intermediate scores as compared to Negroes. The distributions of the five-minute scores by race show no essential difference.

There is a strong association of both one and five-minute scores with birthweight, with low birthweight associated with low Apgar scores. There is also a slight trend to lower Apgar scores at both one and five minutes for the heavy infants with birthweights in excess of 4000 grams.

There is a strong observed association of Apgar score with neonatal mortality. The distribution of neonatal mortality by one-minute Apgar score fits precisely that reported by Apgar and James in October 1962, which was based on 27,715 cases.

The stronger relationship between the five-minute score and neonatal mortality, compared to that of the one-minute score, suggests that the five-minute score may be a valuable addition to the one-minute Apgar

in predicting neonatal mortality and identifying high risk groups.

If birthweight, as well as Apgar score is considered, there is a measurable increase in this predictive value.

Part B Included: Yes.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publication: Drage, J. S., M.D., Kennedy, C., M.D., Schwarz, B. K., L.L.B.: The Apgar Score as an Index of Neonatal Mortality. Obstet. Gynec. 24:2, 222-230, Aug. 1964

Serial No. NDB-(CF)-63 PR/PN 1155
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Apgar Score - An Index of Infant Morbidity.

Principal Investigators: Joseph S. Drage, M.D., PRB, NINDB
Benjamin K. Schwarz, PRB, NINDB
Charles Kennedy, M.D., Children's Hospital of Philadelphia

Cooperating Units: Children's Hospital of Philadelphia, Pennsylvania

Man Years:	F.Y. 1965
Total:	4/12
Professional:	3/12
Other:	1/12

Project Description: This project relates Apgar scores taken at one and five minutes after birth and birthweight to certain indices of infant morbidity based on a neurological examination at one year of age. Outcomes at one year are defined in terms of overall neurological abnormality of muscle tone, locomotive retardation, and abnormalities of prehensile grasp. A general summary of the preliminary tabulation shows that this data gives support to previous reports in the literature that relate low birthweight to abnormal neurological outcome, not only in terms of overall diagnostic judgment, but in terms of specific judgments regarding locomotor development, muscle tone and prehensile grasp. In the design of this study and in terms of the definitions of neurological outcome at one year, this preliminary analysis of project data suggests that the five-minute score has value in predicting infant morbidity and that this predictive ability is independent of birthweight. Within the combinations of birthweight in five-minute score groups, abnormal neurological outcome is associated more strongly with the combination of low birthweight and low five-minute score. In general, the percent abnormal within each birthweight group is greater among those infants with scores of 0-3 than among those infants in the same birthweight group with scores of 7-10. It appears that while both birthweight and five-minute scores have predictive ability regarding neurological abnormality at one year,

this predictive ability increases considerably when birthweight and five-minute scores are combined.

Study in progress.

Part B Included: No.

Serial No. NDB-(CF)-63 PR/PN 1156
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Neonatal Hyperbilirubinemia and Subsequent Neurological Sequelae.

Principal Investigators: Janet Hardy, M.D., Johns Hopkins Hospital
T. R. Boggs, M.D., Pennsylvania Hospital
Heinz Berendes, M.D., PRB, NINDB
B. H. Williams, M.D., PRB, NINDB
William Weiss, PRB, NINDB
Joseph S. Drage, M.D., PRB, NINDB

Cooperating Units: Pennsylvania Hospital, Philadelphia, Pa.
Perinatal Research Branch, NINDB

Man Years:	F.Y. 1965
Total:	2/12
Professional:	1/12
Other:	1/12

Project Description: During the past year, the investigators have redesigned the bilirubin study based on the information gained from the first set of tabulations. Tabulations have been obtained from the computer and the graphs, tables and charts emanating from this data have been distributed to the above-listed investigators for their analysis. It is anticipated that this study will clarify the relationship of bilirubin levels between 10-20 mg.% and neurological sequelae.

Study in progress.

Part B Included: No.

Serial No. NDB-(CF)-63 PR/PN 1159
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Aplasia of One Umbilical Artery.

Principal Investigators: W. A. Blanc, M.D., Columbia University
Jerome Deutschberger, PRB, NINDB
Lenore Bajda, M.D., PRB, NINDB
Luz A Froehlich, M.D., PRB, NINDB
Toshio Fujikura, M.D., PRB, NINDB
B. H. Williams, M.D., PRB, NINDB

Cooperating Units: Columbia University College of Physicians and Surgeons, New York City, New York
Perinatal Research Branch, NINDB

Man Years:	F.Y. 1965
Total:	1/12
Professional:	.5/12
Other:	.5/12

Project Description: A total of 26,539 placentas of single births obtained from 12 different institutions enrolled in the Collaborative Study were evaluated, in which 203 (0.76%) showed a single umbilical artery on gross and microscopic examination.

The incidence of single umbilical artery in the White was 1.22%, a higher incidence than in the Negro, which was 0.44%. This racial difference was consistently present in the various institutes and in all maternal age and birthweight groups. Conversely, the incidence of associated congenital malformations was higher in the Negro (42.1%) than in the White (23.0%).

Associated congenital malformations were classified into three types: A (lethal or major), B (non-lethal), and C (borderline) malformations. The overall incidence of associated malformations (Types A, B, plus C) was 28.6% or 48 of the 203 infants. When the Type C malformations were excluded, the incidence was 16.7%. Of 99 infants who had record of a 12-month follow-up examination, the incidence of congenital malformations exclusive of the Type C group was 10 (10.1%). Of the 203 infants, twenty (9.8%) were stillborn. Six (3.3%) died during the neonatal

period and 3 (1.6%) died in infancy. In 10 (50.0%) of the 20 stillbirths, associated malformations were present, and in 9 (45.0%) of these, the malformations were Type A. Five of the 6 who died during the neonatal period showed congenital malformations, and 4 of these had Type A malformations. Only 7 (4.0%) of the 174 survivors showed Type A malformations. Skeletal system anomalies showed the highest incidence in the associated malformations, and of these the most frequently encountered were polydactyly (3.4%), and vertebral malformations (2.9%). All organ systems were involved in the associated malformations except possibly the endocrine system.

Of the 203 infants, 93 (45.8%) were male and 110 (54.2%) female. The incidence of SUA was 1.98% in infants of low birthweight (2500 grams and under). In infants weighing over 2500 grams, the incidence was 0.63%.

The incidence of placental weights under 300 grams was higher in the SUA placentas (13.9%) than in the controls (3.7%). Twelve or 5.9% of the 203 umbilical cords showed velamentous insertion, whereas that of the control group was 422 or 2.07% of 20,366. Maternal diabetes was 6.4%, an incidence 5 times higher than in the control group.

Study in progress.

Part B Included: Yes.

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B: Honors, Awards, and Publications

Publication: Froelich, L. A., M.D., Fujikura, T., M.D.: Aplasia of One Umbilical Artery. Am. J. Ob. Gyn. In press.

Serial No. NDB-(CF)-63 PR/PN 1161
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Prematurity.

Principal Investigators: Milton C. Westphal, M.D., Children's Hospital, Buffalo, N. Y.
Edward H. Bishop, M.D., Pennsylvania Hospital
B. H. Williams, M.D., PRB, NINDB
Heinz Berendes, M.D., PRB, NINDB
Jerome Deutschberger, PRB, NINDB
Benjamin K. Schwarz, PRB, NINDB

Cooperating Units: Pennsylvania Hospital, Philadelphia, Pennsylvania
Perinatal Research Branch, NINDB

Man Years:	F.Y. 1965
Total:	3/12
Professional:	2/12
Other:	1/12

Project Description: The objectives of a prematurity study are the exploration of a combination of birthweight and gestational length as a more sensitive, graded response predictor of unfavorable outcome than these factors taken separately; to assess the strength of the association of this combination with certain major recognized unfavorable outcomes, such as death, congenital malformations, mental deficiency, and certain specific neurological disease states; to determine those lesser characteristics of morbidity which differentiate gravidae in high-risk groups from those with normal expectation of successful pregnancy; to determine those factors around the period of pregnancy which serve to differentiate gravidae into the various high-risk groups, and to quantitate these risks (examples of these factors are race, sex, age, parity, etc.); to determine those factors which also serve to differentiate gravidae into high-risk groups, but which must be assessed in more complex situations (examples of these factors would be toxemia, medical complications, placenta previa, etc.).

Study in progress.

Part B Included: No.

Serial No. NDB-(CF)-63 PR/PN 1162
1. Perinatal Research Branch
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Apgar Scores.

Principal Investigators: Joseph S. Drage, M.D., PRB, NINDB
William Weiss, PRB, NINDB
Benjamin K. Schwarz, PRB, NINDB
Charles Kennedy, M.D., Children's Hospital of Philadelphia

Cooperating Units: Children's Hospital of Philadelphia, Pennsylvania

Man Years:	F.Y. 1965
Total:	4/12
Professional:	3/12
Other:	1/12

Project Description: This project will match the one and five-minute scores of each child and will relate the various combinations to outcome, considering the resuscitative procedure used. Such studies should sharpen the apparent relationship between the combined use of the one and five-minute scores and their relationship with outcome in terms of mortality and morbidity. In addition, prenatal factors will be related to scores and the scores in turn to outcome. Infants with low scores associated with a specific prenatal factor may be more strongly correlated to undesirable outcome than equally low scores unassociated with such a prenatal factor. In other words, the significance of a low Apgar Score as a predictor of mortality and morbidity may depend on the etiology of the low score in terms of specific events or conditions during pregnancy, labor and delivery.

Study in progress.

Part B Included: No.

Serial No. NDB-(CF)-63 PR/PN 1163
1. Perinatal Research Brancy, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: An Investigation Into the Relationship Between Congenital Heart and Great Vessel Anomalies and Selected Perinatal Factors as Recorded in the Collaborative Perinatal Research Project.

Principal Investigators: Lenore Bajda, M.D., PRB, NINDB
Sheila Mitchell, M.D., O.A.D.C.S.

Cooperating Units: Laboratory of Technical Development, OADCS, NHI, Bethesda, Maryland

Man Years:	F.Y. 1965
Total:	2/12
Professional:	1/12
Other:	1/12

Project Description: The primary objectives of the study are to assess the relationship between certain perinatal factors and congenital heart-great vessel anomalies; factors considered include maternal antibody titre responses to selected viral antigens and maternal blood groups.

Secondary objectives include investigating relationships between early signs of abnormality and congenital heart-great vessel anomalies and relationships between the existence of a congenital heart-great vessel anomaly and mental retardation as recorded in the eight-month psychological examination and the one-year summary records.

Study data is obtained from Collaborative Study records received by PRB from the onset of the Study (January 1959) to December 1964. The Study group is drawn from a population pool of approximately 32,000 live and stillbirth reports received during this period.

Study in progress.

Part B Included: No.

Serial No. NDB-(CF)-63 PR/PN 1164
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Early Signs of Neurological Abnormality in Newborn Infants.

Principal Investigators: Joseph S. Drage, M.D., PRB, NINDB
Karin B. Nelson, M.D., PRB, NINDB
B. H. Williams, M.D., PRB, NINDB
Sheldon Korones, M.D., University of Tennessee
Charles Kennedy, M.D., Children's Hospital of Philadelphia

Cooperating Units: University of Tennessee, Memphis, Tennessee
Children's Hospital of Philadelphia, Philadelphia, Pa.

Man Years:	F.Y. 1965
Total:	4/12
Professional:	3/12
Other:	1/12

Project Description: This study will attempt to define constellations of abnormal neurological findings reported during the newborn nursery period and relate these constellations to abnormal neurological outcome at eight and twelve months. The associations should consider the established relations between abnormal neurological outcome at one year, birthweight and Apgar score.

The predictive ability of the constellation may be increased by weighing various findings within the constellation, or by including within the constellations abnormality often associated with neurological abnormality such as cardio-respiratory abnormality.

Study in progress.

Part B Included: No.

Serial No. NDB-(CF)-63 PR/PN 1165
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Clefts of Lip and Palate as Indicators of Perinatal Morbidity.

Principal Investigators: R. Sleeter, M.D., University of Oregon Medical School
Lenore Bajda, M.D., PRB, NINDB

Cooperating Units: Perinatal Research Branch, NINDB

Man Years:	F.Y. 1965
Total:	2/12
Professional:	1/12
Other:	1/12

Project Description: An attempt will be made to correlate genetic history and other selected prenatal factors to the incidence of Cleft Lip and/or Palate in the Collaborative Study Population. Study in abeyance until the Collaborative Study records can provide a greater number of cases for analysis.

Study in progress.

Part B Included: No.

Serial No. NDB (CF) -65 PR/PN 1270
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Toxoplasmosis: Serological and Clinical Studies.

Principal Investigators: John L. Sever, M.D., PRB, NINDB
Joseph S. Drage, M.D., PRB, NINDB

Cooperating Units: Perinatal Research Branch

Man Years:	F.Y. 1965
Total:	2/12
Professional:	1/12
Other:	1/12

Project Description: This study relates rises in antibody titer to abnormal pregnancy outcomes. Within the Pediatric Neurology Section, a hand review was completed on 128 cases that had shown various degrees of titer rise. Within the group of 47 patients with titer elevations of greater than 4096, or significant increases in antibody titer, five were found to have definite toxoplasmosis and ten were suspected of having toxoplasmosis. The ten included six with motor retardation, two pregnancies resulted in stillbirths, and two in neonatal deaths. The sera from ten of the remaining 32 apparently normal children were tested for antibody to toxoplasmosis and one was found to have a high titer.

Study in progress.

Part B Included: No.

Serial No. NDB (CF)-65 PR/PN 1271
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Maternal Rubella and Pregnancy Outcome.

Principal Investigators: John L. Sever, M.D., PRB, NINDB
Karin B. Nelson, M.D., PRB, NINDB

Cooperating Units: Perinatal Research Branch, Section on Infectious Diseases, NINDB
Perinatal Research Branch, Section on Pediatric Neurology, NINDB

Man Years:	F.Y. 1965
Total:	2/12
Professional:	1/12
Other:	1/12

Project Description: Analysis is in progress, in conjunction with the Section on Infectious Diseases, of clinical records of infants born to women exposed to rubella in the 1964 epidemic. 6161 pregnant women were under study during the first six months of 1964. Of these, 2434 (38%) were exposed to rubella, 668 (10.8%) of them in the first trimester. Clinical rubella developed in 135 women; in 52 in the first trimester and in 83 in the second and third trimesters.

Records are now available for 51 of the 52 infants born to mothers who had clinical rubella in the first trimester. There are five definite and one suspected cases of rubella syndrome in these children. Among infants born after exposure in the second and third trimesters, two are abnormal and at least two more have shown hearing defects. This data probably represents a minimal estimate of rubella-related damage, because clinical evidence is thus far available only to the time of nursery discharge in most instances. Other studies have shown that defects may become apparent only in later infancy and childhood. Therefore, it is necessary to continue follow-up of the Study sample before the true extent of defect due to rubella can be evaluated.

Records of approximately 75% of the infants whose mothers were exposed to rubella in the first trimester, but did not develop clinical rubella,

are now available for analysis through the newborn period. Work in progress shows that while only one instance of the rubella syndrome has been observed in this group, rates of prematurity (by weight) and stillbirth are considerably above Study averages. Presumably inapparent rubella has occurred in some mothers of this group. The correlation of serologic testing with clinical results, as is now being undertaken, should permit valuable estimates of the frequency with which exposure without apparent disease in pregnant women leads to fetal damage.

Study in progress.

Part B Included: No.

Serial No. NDB (CF)-65 PR/PN 1272
1. Perinatal Research Br[illegible]h, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Hyaline Membrane Disease.

Principal Investigators: James Drorbaugh, M.D., Children's Medical Center, Boston
William Cochran, M.D., Boston Lying-In Hospital
B. H. Williams, M.D., PRB, NINDB
Toshio Fujikura, M.D., PRB, NINDB
Benjamin K. Schwarz, PRB, NINDB

Cooperating Units: Perinatal Research Branch, NINDB

Man Years:	F.Y. 1965
Total:	3/12
Professional:	2/12
Other:	1/12

Project Description: This study is evaluating maternal characteristics, socio-economic data, factors of pregnancy, labor, and delivery, in terms of respiratory distress syndrome (hyaline membrane disease) in the pregnancy outcome. Study cases identified as respiratory distress syndrome have been obtained from the PED-8 (Newborn Diagnostic Summary). This group of cases would include false positive; that is, cases carrying the diagnosis but without x-ray and laboratory data to justify such a diagnosis. Identification of the false negatives must also be made; cases with the syndrome having laboratory and x-ray evidence of having the syndrome, but not reported on the PED-8, will be identified by reviewing all cases that have received oxygen therapy as reported on the PED-3. The definition of the respiratory distress syndrome is: Increased respiratory rate, retractions, cyanosis, decreased pH, increased CO_2, and x-ray evidence compatible with the diagnosis.

Once the Study cases have been satisfactorily identified and reviewed, correlations will be made relating maternal characteristics, factors of labor and delivery, and socio-economic data to the mortality and morbidity of the offspring. Special interest has been shown in the

neurological morbidity of those infants surviving the syndrome. Study in progress.

Part B Included: No.

Serial No. NDB-(CF)-63 PR/BS 1166
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Evaluation of the Gross and Fine Motor Functions of Children Eight-Months of Age in Relation to the Neurological Results of the Examination of the Children at One-Year of Age

Principal Investigator: Dr. Martin A. Mendelson

Other Investigators: Dr. Emmy E. Werner

Cooperating Units: Collaborative Institutions, COLR Project, NINDB (Data from records)

Section on Pediatric-Neurology, PRB, NINDB

Man Years (Computed for 18-month period):
Total: .5 man years
Professional: .2 man years
Other: .3 man years

Project Description:

The objective of this study is to determine whether the gross and fine motor performance of children, assessed at 8-months of age, is related to the results of neurological examinations given to the same children at one-year of age. It is hypothesized that the degree of dysfunction in fine and gross motor performance will be indicated correspondingly in the degree of dysfunction, as well as the type found neurologically in children at one-year of age.

These studies are in progress; they have not been processed sufficiently as yet on account of delays in acquiring computer facilities and time.

Part B Included No

Serial No. NDB-(CF) PR/BS 1167
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Explorative Study for the Use of a Speech and Language Screening Examination for 3-Year-Old Children in the Home Situation

Principal Investigator: Dr. Miriam F. Fiedler

Other Investigators: Dr. Martin A. Mendelson

Cooperating Units: Section on Behavioral Sciences, PRB, NINDB

Section on Pediatric-Neurology, PRB, NINDB

Man Years (Computed for 18-month period):
Total: .5 man years
Professional: .2 man years
Other: .3 man years

Project Description:

This is the first of two studies to try and determine the most efficient way of identifying those 3-year-olds whose speech and language development is atypical and may be associated with neurological and sensory defects. At Boston, the Children's Medical Center has developed a home interview form designed to obtain information on the child's speech, language and hearing from the mother; a comparative study of children whose mothers have been interviewed with this form, and on whom (the children) speech, language and hearing examination results are available, is contemplated.

These studies are in progress; they have not been processed sufficiently as yet on account of delays in acquiring computer facilities and time.

Part B included No

Serial No. NDB-(CF)-63 PR/BS 1168
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Indexes of Socio-Economic Status of Study Mothers in Relation to Outcomes of Pregnancy

Principal Investigator: Dr. Martin A. Mendelson

Other Investigators: None

Cooperating Units: Section on Epidemiology and Genetics, PRB, NINDB

Section on Obstetrics, PRB, NINDB

Section on Pediatric-Neurology, PRB, NINDB

Man Years (Computed for 18-month period):

Total:	.8 man years
Professional:	.5 man years
Other:	.3 man years

Project Description:

This study involves an attempt to determine the relationship between specific socio-economic indexes and specific outcomes of pregnancy. It is hypothesized that prenatal morbidity and mortality will increase as the socio-economic index decreases.

These studies are in progress; they have not been processed sufficiently as yet on account of delays in acquiring computer facilities and time.

Part B included No

Serial No. NDB-(CF)-63 PR/BS 1169
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Antecedents and Correlates of Retarded Motor Development

Principal Investigator: Dr. Audrey Shechtman

Other Investigators: Dr. Martin A. Mendelson

Cooperating Units: Collaborative Institutions, COLR Project, NINDB (Data from records)

Section on Pediatric-Neurology, PRB, NINDB

Section on Behavioral Sciences. PRB, NINDB

Man Years (Computed for 18-month period):
Total: .8 man years
Professional: .4 man years
Other: .4 man years

Project Description:

The major objective of this study is to investigate the variables on which 8-month-old infants judged to be normal, suspect, or abnormal in motor development, in terms of Bayley Motor Scale norms, can be differentiated neonatally at the 4 and 8-month levels. Additional objectives are: (1) to determine the level of functioning of these groups on the Bayley Mental Scale; and (2) to explore relevant behavioral variables and their relationships to the above.

These studies are in progress; they have not been processed sufficiently as yet on account of delays in acquiring computer facilities and time.

Part B included No

Serial No. NDB-(CF)-63 PR/BS 1170
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Socio-Economic Status Groupings and Their Relationship to Illnesses and Disorders as Reported on the OB-3, "History Since Last Menstrual Period," and OB-8, "Repeat Prenatal History"

Principal Investigator: Dr. Martin A. Mendelson

Other Investigators: Dr. Rudolph Vollman

Cooperating Units: Section on Epidemiology and Genetics, PRB, NINDB

Section on Obstetrics, PRB, NINDB

Collaborative Institutions, COLR Project, NINDB (Data from records)

Man Years (Computed for 18-month period):

Total:	.5 man years
Professional:	.3 man years
Other:	.2 man years

Project Description:

The hypothesis of this study assumes that the number of symptoms of disorders reported by Study gravidae is related to their socio-economic status in such a way that the greater the number of illnesses or discomforts reported, as well as the type of illnesses or discomforts reported, increase, as the socio-economic status of the gravidae decreases. Information from OB-3 and OB-8 (History Since LMP and Repeat Prenatal History) are the major dependent variables.

These studies are in progress; they have not been processed sufficiently as yet on account of delays in acquiring computer facilities and time.

Part B included No

Serial No. NDB-(CF)-63 PR/BS 1171
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: An Evaluation of the Offspring of Unwed Mothers in Terms of the Complications of Birth and Delivery, Results on the 4-Month Examination, 8-Month Examination and One-Year Examination, as Compared to a Matched Group of Married Mothers and Their Offspring

Principal Investigator: Dr. Martin A. Mendelson

Other Investigators: None

Cooperating Units: Section on Pediatric-Neurology, PRB, NINDB

Section on Obstetrics, PRB, NINDB

Collaborative Institutions, COLR Project, NINDB (Data from records)

Man Years (Computed for 18-month period):
Total: .6 man years
Professional: .4 man years
Other: .2 man years

Project Description:

The purpose of this study is to determine whether there are significant differences between the offspring of a selected group of unwed mothers as compared to a matched group of married mothers. There is reason to believe, based on previous research, that the unwed mother is subject to various cultural and psychological stresses that can effect the course of her pregnancy, may show up in complications of birth and delivery, and may have measurable influences on the development of the offspring.

These studies are in progress; they have not been processed sufficiently as yet on account of delays in acquiring computer facilities and time.

Part B included No

Serial No. NDB (CF)-65 PR/BS 1273

1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A:

Project Title: Hypothesized Performance of 8-Month-Old Children, Considered Premature at Birth, on the COLR Form of the Bayley Scales of Mental and Motor Development.

Principal Investigators: Dr. Heinz Berendes, Dr. Martin Mendelson

Other Investigators: None

Cooperating Units: None

Man Years (Computed for 18-month period):
Total: .5 man years
Professional: .2 man years
Other: .3 man years

Project Description:

The objective of this investigation is to follow-up a premise originating from the studies of Drs. Pasamanick and Knobloch regarding postnatal performance of premature children on objective scales of mental and motor performance. It has been indicated that it may not be fair to judge, at least during the first years of life, the premature infant's performance on psychological instruments that have been developed on the basis of age-level norms.

Computer time has been obtained for this project and tabulations of data have been made.

Part B included No

Serial No. NDB (CF)-63 PR/EG 1172
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Pregnancy Outcome and Prior Pregnancy Record

Principal Investigators: Dr. V. Elving Anderson, Univ. of Minnesota
Dr. N. C. Myrianthopoulos, PRB, NINDB
Paul L. Canner, Univ. of Minnesota

Cooperating Units: University of Minnesota

Man Years
Total: .25
Professional: .25

Project Description:

This Study is being continued to examine the extent to which information about the course and outcome of prior pregnancies of the gravida may be used to predict the outcome of the current pregnancy, and to look for important biological variables affecting reproductive wastage.

Preliminary analysis showed some differences among racial or ethnic groups; the proportion of first trimester registrants for white was about double that for Negro and Puerto Rican women. The risk of abortion in the Study pregnancy was highest for those gravidae with a prior abortion. Prior stillbirth also appeared to increase the risk of neonatal death, and vice versa. With respect to birthweight, when the last prior child weighed 2000 grams or less, a subsequent child had a 37.4 percent chance of weighing 2500 grams or less. The proportion of Study pregnancies with products weighing less than 2500 grams was higher in Negroes than in whites.

Present Status:

The first stage of multivariate analysis has been designed and a request for the necessary data has been submitted to the Section on Systems, Design and Procedures.

Part B included No

Serial No. NDB (CF)-63 PR/EG 1173
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: A Study of Socioeconomic, Medical and Genetic Factors in Major Congenital Malformations

Principal Investigators: Dr. N. C. Myrianthopoulos, PRB, NINDB
Dr. L. Bajda, PRB, NINDB; Dr. C. Chung, NIDR

Man Years
Total: .25
Professional: .25

Project Description:

This is a continuation of a project to study the relation of medical and genetic factors on major congenital malformations. Single malformations are selected on the basis of their frequency from births registered with the Collaborative Study. These are: anencephaly, polydactyly, cleft palate and/or harelip, spina bifida, and meningocele, congenital heart defects and hypospadias. Each malformation is controlled by two normal births from the same sex, racial group and Institution. The malformations and controls are studied with respect to birth order, age of parents, several socioeconomic factors, pregnancy illnesses and complications, and family history.

Present Status:

The study is now being programmed for multiple regression analysis.

Part B included No

Serial No. NDB (CF)-63 PR/EG 1174
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Birthweight in Relation to Selected Socioeconomic Variables

Principal Investigators: Dr. N. C. Myrianthopoulos, PRB, NINDB

Other Investigators: Dr. A. F. Naylor, PRB, NINDB

Man Years

Total:	.45
Professional:	.30
Other:	.15

Project Description:

A study is being continued to relate birthweight to socioeconomic variables such as family income, parental education and occupation, housing density and other information gathered in Study interviews. The two-fold object continues to be to treat birthweight as a significant parameter in itself and to attempt to develop a single socioeconomic index with biological meaning in the study of other parameters like malformation frequency and abortion frequency.

Present Status:

A partial sample study using a card sorter has suggested that some curvilinear relationships exist. A multivariate covariance analysis of the race variable is being undertaken. Within race, "stepwise" regression analysis will be performed. A combined program for both such analysis has been brought to an advanced stage of development through the efforts of SSDP and the NIH Computation Branch.

Part B included No

Serial No. NDB (CF)-63 PR/EG 1175
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Determination of the Zygosity of Twins Born to Mothers in the Collaborative Study

Project Coordinator: Dr. N. C. Myrianthopoulos, PRB, NINDB

Cooperating Units: All Institutions participating in the Collaborative Study

Man Years
Total: .25
Professional: .25

Project Descriptions:

This is a continuation of a project to determine the zygosity of twins born to Study mothers. This determination is basic and vital to any subsequent studies using twins. Twin zygosity is determined by comparison of sex, placentation, blood groups, and finger and palm prints. This information is forwarded by all Institutions to the Section on Epidemiology and Genetics where it is classified and analyzed by special methods. The results are kept on file and are available to anyone wishing to engage in twin research.

Present Status:

In progress.

Part B included No

Serial No. NDB (CF)-63 PR/EG 1176
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: A Genetic and Socioeconomic Study of Habitual Aborters

Principal Investigators: Dr. N. C. Myrianthopoulos, PRB, NINDB

Other Investigators: Dr. A. F. Naylor, PRB, NINDB

Man Years
Total: .20
Professional: .20

Project Description:

This is a continuation of prospective Study of gravidae whose prior pregnancy history shows three or more early or late fetal deaths, excluding known induced abortions. The objective is to determine if genetic and/or socioeconomic factors are related to habitual abortions. Study gravidae are matched with controls by same number of prior pregnancies but no more than one fetal death, Institution, race and age. Study variables include: outcome of first Study pregnancy, outcome of subsequent pregnancies where available, consanguinity on two levels, known cause of repeated abortions, reproductive history of sisters in Study where available, and socioeconomic factors.

Present Status:

Preliminary listings of data needed for the design and programming of the analysis have been received and work is in progress.

Part B included No

Serial No. NDB (CF)-63 PR/EG 1177
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Genetic and Socioeconomic Factors in Early and Late Fetal Death

Principal Investigator: Dr. N. C. Myrianthopoulos, PRB, NINDB

Other Investigators: Dr. A. F. Naylor, PRB, NINDB
Blanche Vincent, PRB, NINDB

Man Years

Total:	.30
Professional:	.20
Other:	.10

Project Description:

This is a continuation of a retrospective Study of gravidae whose first Study pregnancy terminated in early or late fetal death. The objective is to determine what effect do genetic and socioeconomic factors have on pregnancy wastage and if a distinction can be made etiologically between early and late fetal death. Study gravidae are matched with controls by Institution, race and age. Study variables include prior pregnancy history, parity, socioeconomic factors, and subsequent pregnancy history, where available.

Present Status:

Preliminary listings of data needed for the design and programming of the analysis have been received and work is in progress.

Part B included No

Serial No. NDB (CF)-63 PR/EG 1178
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: A Socioeconomic Index for the Population of the Collaborative Project

Principal Investigator: Dr. N. C. Myrianthopoulos

Other Investigators: Anne Kantor, PRB, NINDB

Man Years

Total:	.40
Professional:	.30
Other:	.10

Project Description:

This is a continuation of a project to describe the socioeconomic status of the Study population by a composite socioeconomic index, from information obtained about the family of the gravida before the Study child is born. The main components of the index are: education, occupation, housing density, marital status, and income. The index is derived for the whole population and by individual Institutions, in two ways: first, by using available U. S. Census scores for education, occupation and income and supplementing by scoring housing density and marital status in the same way. This index will enable us to compare socioeconomically the Study population with the United States population. Second, by deriving empirical scores for the socioeconomic variables from the distribution of these variables in the Study population. This will enable us to obtain an objective socioeconomic picture of our population and observe similarities and differences among the various participating Institutions.

Present Status:

The project is essentially completed. The two indices have been derived with a slight departure from the original plan, i. e. using only education, occupation and income. The derived scores and indices are now being put on tape for every case and should be available for use very soon.

Part B included No

Serial No. NDB (CF)-63 PR/Eg 1179
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Association of Blood Groups to Amyotrophic Lateral Sclerosis

Principal Investigators: Dr. N. C. Myrianthopoulos, PRB, NINDB

Other Investigators: P. Schmidt, CC
W. Leyshon, NIDR

Cooperating Units: Blood Bank, CC, Genetics Branch, NIDR

Man Years
Total: .20
Professional: .20

Project Description:

A continuation of pilot study to determine if there exists any selection for a specific blood type among patients with motor neurone disease.

Present Status:

The study is now completed. Analysis of the material shows a very slight association with the P group but considerable association with the Secretor factor. The material is being prepared for publication.

Part B included No

Serial No. NDB (CF)-63 PR/EG 1180
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: A Survey of Schizophrenia Among the Relatives of Schizophrenic Patients

Principal Investigator: Dr. N. C. Myrianthopoulos, PRB, NINDB

Cooperating Units: Spring Grove State Hospital
St. Elizabeth's Hospital

Man Years
Total: 0
Professional: 0
Other: 0

Project Description:

A Study to determine the occurrence of schizophrenia among the relatives of patients who have already been selected for another project (Parkinsonism - ataraxic drugs) and to determine the role of inheritance in schizophrenia. This project being a by-product of another project, has the advantage of overcoming some of the biases involved in selecting an adequate sample. The collection of data from Spring Grove State Hospital has been completed. These will be implemented with data from St. Elizabeth's Hospital concurrently with the project on ataraxic drugs conducted there before genetic analysis is attempted.

Present Status:

No further progress has been made.

Part B included No

Serial No.: NDB (CF)-63 PR/EG 1183
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: A Genetic Study of Parkinsonism and the Dystonias Produced by Ataraxic Drugs

Principal Investigator: Dr. N. C. Myrianthopoulos, PRB, NINDB

Other Investigators: Francis Waldrop
Blanche Vincent

Cooperating Unit: St. Elizabeth's Hospital

Man Years

Total:	0
Professional:	0
Other:	0

Project Description:

A Study to confirm earlier findings. If the impression is confirmed that there is an hereditary susceptibility to Parkinsonism produced by phenothiazine derivatives then the effects of phenothiazines will be studied in those siblings and close relatives of patients and controls who have mental illness and are institutionalized. The known degree of relationship of relatives to patient will serve as a basis for prediction of the eventual outcome if susceptibility to ataraxic drugs is inherited along Mendelian lines.

Present Status:

No further progress has been made.

Part B included No

Serial No. NDB (CF)-63 PR/EG 1184
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Population Dynamics of Tay-Sachs' Disease

Principal Investigator: Dr. N. C. Myrianthopoulos, PRB, NINDB

Other Investigators: Dr. Stanley Aronson, State University of New York

Man Years

Total:	.30
Professional:	.20
Other:	.10

Project Description:

A Study to determine whether differential fertility favoring the Jewish heterozygote can account for the 100-fold higher frequency of Tay-Sachs' disease and the gene responsible for it among the Jewish compared with non-Jewish population in the U. S.

Present Status:

The collection of fertility data for presumed Jewish and non-Jewish heterozygotes and also for adequate controls, has been completed. Preliminary analysis indicates that there might be a reproductive advantage to the Jewish heterozygote as compared to the homozygous normal. A more sophisticated analysis is now being performed to confirm this finding.

Part B included No

Serial No. NDB (CF)-65 PR/EG 1274
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Genetic Bases of Neonatal Reflexes

Principal Investigator: Dr. A. F. Naylor, PRB, NINDB

Other Investigators: Dr. N. C. Myrianthopoulos, PRB, NINDB

Man Years
Total: .25
Professional: .20
Other: .05

Project Description:

Inquiry is made into the possible validity of regarding the suck, rooting and other neonatal reflexes as fairly definite genetic entities. If this is so, loss of reflex function should occur as the result of genetic processes like assortment, segregation and mutation; losses due to these processes might occur in cases which are not accountable in terms of prematurity or intra-uterine environment.

Present Status:

A request has been sent to the Section on Systems, Design and Procedures for a preliminary listing of certain kinds of reflex loss observed in Collaborative Study babies.

Part B included No

Serial No. NDB (CF)-65 PR/EG 1275
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Study of Family Size with Respect to Rh Blood Type and Other Variables

Principal Investigator: Dr. A. F. Naylor

Other Investigators: Dr. N. C. Myrianthopoulos

Man Years

Total:	.30
Professional	.20
Other:	.10

Project Description:

The project will re-examine the suggestion made by Glass in 1950 that fetal loss in Rh negative women may actually be over-compensated for in a population practicing birth control, which may be lifted when incompatibility loss occurs. Information on the Rh type of gravidae, previous incompatibility complications, family planning practices and race will be related to age-corrected family sizes of gravidae.

Present Status:

A request for appropriate data processing has been sent to the Section on Systems, Design and Procedures.

Part B included No

Serial No. NDB (CF)-65 PR/EG 1276
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Sequential Aspects of Occurrence of Spontaneous Abortion in Family Histories

Principal Investigator: Dr. A. F. Naylor, PRB, NINDB

Man Years
Total: .30
Professional: .25
Other: .05

Project Description

Collaborative Project data are used to check whether abortion frequency has true age or parity relations and/or whether abortions tend to cluster in the reproductive histories of multiply-aborting women. The question of a "true" age-dependence is important especially since James (1963) suggested that an artifactual or spurious age correlation could arise if women who are innately more likely to abort, independently of age at conception, continue to attempt to satisfy family-size aspirations through later ages when non-aborters have restricted their reproductive activities.

Present Status:

Since the statistical methodology is not standard and since the nature of the data to be used (especially the OB-2 records) require exceedingly detailed specification in data processing, the principal investigator has undertaken to write the computer program for the project. It is anticipated that the results will be available about the time this report is submitted.

Part B included No

Serial No. NDB(CF)-65 PR/EG 1277
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: A Study of the Properties of B. Woolf's Statistic for Combined Analysis of 2 X 2 Tables

Principal Investigator: Dr. A. F. Naylor, PRB, NINDB

Man Years

Total:	.10
Professional:	.09
Other:	.01

Project Description:

As an outgrowth of methodological discussions within the Section, attention has been directed to the problem of combining tests of pairs of samples from binomial populations. The problem arises widely in the treatment of epidemiological data. B. Woolf (1955) has suggested a statistic for the purpose of making a combined test and stating the strength of association in a two-fold classification of discrete data. The statistic is a fairly new one when its generality of application is considered and its mathematical properties have not been exhaustively considered.

Present Status:

Relatively brief (15 minutes running time) computer investigations programmed by the Principal Investigator have indicated that at least by one important criterion the old fashioned statistic (in its strictly correct form) which compares the difference of two proportions to the estimated standard error of the difference is superior to Woolf's statistic for hypothesis testing. The results need a little refinement before they can be published.

Part B included No

Serial No. NDB (CF)-65 PR/P 1278

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Biologic Pattern Data Processing

Principal Investigators: Dr. Lewis E. Lipkin (NIH)
Mr. Russell A. Kirsch (NBS)

Cooperating Units: Artificial Intelligence Group,
National Bureau of Standards

Objectives: To develop plans and specifications for a general purpose device to permit computer acceptance of descriptions, orders and questions by a human neuropathologist in pseudo-natural language, concerning microscopic images, and syntax directed machine processing of analyses so indicated, including reply to questions involving both quantitative and topological classes of image processing.

Methods Employed: Linguistic analysis and microgrammar construction, device simulation on IBM 7094 (NBS), advanced programing techniques in the artificial intelligence area.

Summary of Activities: For purposes of reporting progress, it is convenient to divide the work done during the present period into four categories; the first category being the analysis of images by computer; the second category, the synthesis of images; the third category, the linguistic research connected with the description of images; and the fourth category, the development of supporting research tools and miscellaneous activities.

In the category of the analysis of images, one product has been described in NBS report 8569 by Dr. George A. Moore, "Design for a Preferred Language for the Command of Automatic Analysis of Micrographs." This report describes several programs that have been useful in the analysis of micrographs. These analyses which are at a non-articular level in their treatment of images can also be performed upon biological photographs. The level of analysis however is primarily directed at obtaining simple numerical data from images and is limited to such operations as counting, measuring sizes and areas of objects and in general those non-articulatable properties of patterns which characterize simple images.

Two programs were written for the IBM 7094 in FORTRAN, also for the analysis of simple images. One program is a smoothing or averaging program which enables images quantized by the scanning device in present use to be smoothed for purposes of noise reduction. The other program obtains transformed images by a modified wave propogation type of technique. According to a theory due to Harry Blum, "A Transformation for Extracting New Descriptions of Shape," which was presented at the Symposium on Models for the Perception of Speech and Visual Form, November 1964, certain simple patterns can be characterized by the way they behave under this type of wave propogation transformation. This hypothesis is being tested.

The second category of investigations on the synthesis of images includes several tasks. The first was a study of generative devices for specifying the structure of pictures primarily of a line drawing nature. The interest in line drawings and the synthesis of line drawing pictures comes from the possibility of a machine transformation of continuous tone images such as micrographs into line drawings from which recognition procedures would follow more simply than from the original continuous tone images. One program for the production of such line drawing images was experimented with on the IBM 7094. This "cartooning" program is a modification of one developed in the PAX language by Professor Narasimhan at the University of Illinois. It was shown possible to obtain line drawings of nerve cells in which a degree of syntactic classification of pictorial components in cells was possible. These components, however, do not have structural counterparts in the cells themselves and consequently the particular cartooning program experimented with has limited applications.

Another program written for the 7094 obtains differentiated or derivative images from scanned photographs. A set of photographs of optical serial sections of cortex were subjected to processing by this derivative program. It was demonstrated that the distinction between images in the plane of focus and those outside of the plane of focus in a microphotograph could be distinguished by the differentiating technique used in this program. It was also shown to be possible to perform a three-dimensional serial reconstruction of line drawings obtained by the differentiation program from optical serial sections. Subsequent experiments will attempt to construct three-dimensional reconstructions within the computer which can then be subjected to geometrical transformations and presented in aspects different from those available in the planes of sectioning of the original images.

The original method for presenting images was subject to some improvement. Instead of using a conventional printer and a type font which distinguishes different intensities but distorts the horizontal and vertical scale factors, it was demonstrated that the use of a specially modified printer with equal scale factors and a special type font could produce images for experimental purposes which had higher quality and more similarity to the original photographs notwithstanding the fact that they were produced on the computer output printer.

In the third category of tasks, research of a linguistic nature was done on the description of biological images. The first task in this category represented the largest effort on the project. This effort was to develop a grammar for a part of the professional subdialect used by neuropathologists in describing photographs of tissue. An original version of this grammar is reported in the paper by W. C. Watt, "PLACEBO IV, Rules, Concordance, Sample Computer Generation," NBS Technical Note 255. A subsequent version, "PLACEBO V," is currently being worked on. A report soon to be issued includes a minor addition to the "PLACEBO IV" report. The grammar described in Technical Note 255 is intended to serve as the core of a grammar to be used in interrogating a system for automated aid for neuropathological analysis. The emphasis in the development of this grammar has been on including as much syntactical structure of the underlying language as possible, and upon including structure in such a way that subsequent versions of such a grammar may properly include previous versions in the sense that as the language is expanded to accommodate more functions and perhaps more users, older parts of the language could remain intact. This question of inclusion among grammars and the languages that they specify received some serious study during this period.

Two other studies on microgrammars were made during this period. One resulted in the forthcoming report, "Prerequisites to the Utility of Microgrammars," by W. C. Watt, NBS Technical Note 258, and the other which has not yet been reported is a study of the general properties and desiderata of microgrammars as tools for use in interrogation systems of the kind we study under the present project.

In studying systems in which discourse is about the subject of pictures, it appeared worthwhile to seek out and study a system in which the pictures and the language descriptive of these pictures was of greater simplicity than in the biological case of primary interest here. The subject of this small study was the descriptions of geometric configurations used in cattle brands. A small grammar for both the descriptions and the brands was written.

Still in the area of description of images, some preparatory work was done during this period in arranging materials for informant work with neuropathologists to attempt to elicit from them descriptions of photographs of neuropathological interest.

The last category of tasks in this project were of a supporting research nature to serve as an aid primarily for the above mentioned tasks. Two programs somewhat different in their operation were written to produce concordances for large grammars. The first, written in the COMIT programing language for the IBM 7094 computer, is somewhat slower than the second written in FORTRAN. The FORTRAN concordance program enables a linguist writing a grammar to have available the list of all syntactic categories and their occurrence within the grammar as a debugging tool in writing grammars.

A particularly important machine tool to have available for linguistically directed analyzers is a parser or syntactic analyzer. Three versions of parsers were investigated. The first was flow charted but not programed. The second was written in FORTRAN, and was somewhat faster than the final version also in FORTRAN which accommodates grammars of the form given in the report on "PLACEBO IV." This last parser is nearing completion at present. Some studies were made of parsers written elsewhere and they all proved unsatisfactory for our purposes for one reason or another.

Powerful machine interrogation systems of the type visualized in the present project are sufficiently unconventional and it was deemed worthwhile to study one version of such a system although the system has little interest from the biological standpoint. This is the large MAC time-sharing system. Some experimentation was done with the MAC time-sharing system and a large program in the COMIT language on the MAC system was written for the purposes of generating sentences from a given grammar. The experience gained in operating on-line with such a powerful time-sharing system will prove useful in extrapolating to the behaviour of the kind of biological interrogation system presently under study. A report produced as a result of this investigation was of some interest for improving the MAC system itself.

Finally, in addition to the above research supporting activities, a National Bureau of Standards seminar was conducted on an occasional basis with invited speakers from both within and outside the government speaking on the subject of language and picture processing.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Watt, W. C.: PLACEBO IV-Rules, Concordances, Sample Computer Generation. National Bureau of Standards, Technical Note #255, March 9, 1965.

Watt, W. C.: Prerequisites to the Utility of Microgrammars. National Bureau of Standards, Technical Note #258, April 1965.

Moore, George A.: Design for a Preferred Language for the Command of Automatic Analyses of Micrographs. National Bureau of Standards, Technical Note #8569, 1965.

Hsu, Robert W.: Comments from a MAC Customer. National Bureau of Standards, Internal Memorandum, December 31, 1964.

Presentations delivered which are to be published in subsequent symposia or proceedings:

Lipkin, Lewis E.: Human Aided Computer Analysis of Morphologic Data. NASA Symposium on the Analysis of Central Nervous System and Cardiovascular Data Using Computer Methods. Lorne D. Proctor (Ed.), In Press.

Lipkin, L. E., Watt, W. C., and Kirsch, R. A.: The Analysis, Synthesis and Description of Biologic Images. New York Academy of Sciences Conference on Advances in Biomedical Computer Applications, June 1965.

Serial No. NDB (CF)-65 PR/P 1279
1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Influence of Race and Other Factors on Pulmonary Hyaline Membranes

Principal Investigators: Dr. Luz A. Froehlich (NIH)
Dr. Toshio Fujikura (NIH)

Cooperating Units: All Collaborating Institutions

Project Description: The pulmonary sections of 434 neonatal deaths out of 27,407 livebirths in consecutive deliveries were reviewed, of which 131, or 4.8 per 1,000 livebirths, showed hyaline membranes. The incidence of birthweights below 2,500 grams was nearly twice as high in the Negro as in the white. However, the neonatal death rate was lower in the Negro than in the white for this birthweight group, but higher in the Negro than the white for birthweights over 2,500 grams. The hyaline membrane rate was consistently lower in the Negro for all birthweight groups. These suggest that the Negro is of more advanced maturity than the white at specific birthweights. Pulmonary hyaline membranes do occur in infants weighing over 2,500 grams and constitute 17.5 percent of the total hyaline membrane cases. In this birthweight group moderate and marked membranes were encountered with nearly the same frequency as slight membranes. Infants of very low birthweight (500-1,500 grams) have a much higher incidence of slight than moderate and marked membranes.

In the infants of very low birthweight (500-1,500 grams) with hyaline membranes,more than 80 percent died within the first 24 hours. The heavier babies with hyaline membranes survived longer. In the non-hyaline membrane group of neonatal deaths, more than 50 percent of the cases died within 24 hours regardless of birthweight. In the hyaline membrane group with survival times of less than 24 hours, slight membranes had a higher indicence than moderate or marked membranes in each birthweight group. The reverse was true with survival times of between 24-72 hours.

In the white, the survival time pattern of the hyaline membrane group was similar to that of the non-hyaline membrane group. In the Negro, however, 71.2 percent of the hyaline membrane group were dead within 24 hours, as against 49.4 percent of the non-hyaline membrane group. Conversely, only 1.5 percent of the Negroes in the hyaline membrane group were still alive at 72 hours in contrast to 23.1 percent of the Negroes in the non-hyaline membrane group. This is probably because the majority of Negro babies with hyaline membranes were of extremely low birthweight.

Pulmonary alveolar hemorrhage of moderate or marked degree was frequently seen in association with pulmonary hyaline membranes (46.6 percent). Pneumonia was noted in 25 percent of hyaline membrane cases and the lesion was frequently associated with moderate or marked membranes.

Study in Progress: The results of this study have been approved by the NINDB Editorial Committee and are now before the PRB, NINDB Publications Review Board for approval.

Part B included No

Serial No. NDB (CF)-65 PR/P 1280
1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: Umbilical Cord Inflammation Study

Principal Investigators: Dr. Muyesser Tuncer (U. of Buffalo)
Dr. Ueda (U. of Buffalo)
Dr. Luz A. Froehlich (NIH)

Cooperating Units: Child Development Program,
University of Buffalo

Objectives: (1) To develop a rapid simple method of detecting neutrophilic infiltration in the umbilical cord.

(2) To determine the relationship between neutrophilic infiltration of the cord and perinatal infection.

Summary of Activities: It is a well accepted fact that intrauterine pneumonia is accompanied by acute inflammation of the umbilical cord and fetal membranes in a high percentage of cases. Methods have been devised by other investigators (frozen section of cord, amnion mounts) for rapid diagnosis of placental inflammation, but they are either tedious or require the services of histo-technicians who are not always available on a 24-hour basis in an average hospital. A pilot study was therefore conducted in collaboration with the University of Buffalo to attain the first objective. A simple method was devised consisting of making umbilical cord imprints under sterile conditions, staining the slides (one with Wright's stain and the other with methylene blue), and scanning for neutrophils and micro-organisms. The entire procedure lasted an average of only 15 minutes for each case. To verify the efficacy of the method, sections of cord and placental membranes were obtained from each case, fixed, forwarded to this laboratory, processed and the permanent histologic sections read by Dr. Froehlich, guided by standardized quantitative criteria for evaluating the degree of inflammation. The pilot study revealed a very good correlation between the imprints and histologic sections, and spurred these investigators to pursue and expand the study. The entire study now consists of 1,000 consecutive cases with clinical data of mother and offspring involving 26 variables. All clinical data and imprint results are now duly coded and punched and the histologic evaluations of cords and placentas are nearing completion. Data analysis should proceed within a few weeks.

Part B included No

Serial No. NDB (CF)-65 PR/P 1281
1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part A

Project Title: The Significance of a Single Umbilical Artery

Principal Investigators: Dr. Luz A. Froehlich (NIH)
Dr. Toshio Fujikura (NIH)

Cooperating Units: All Collaborating Institutions

Project Description: A total of 26,539 placentas of single births obtained from 12 different institutions enrolled in the Collaborative Study were evaluated, in which 203 (.76 percent) showed a single umbilical artery on gross and microscopic examination.

The incidence of single umbilical artery in the white was 1.22 percent, a higher incidence than in the Negro, which was .44 percent. This racial difference was consistently present in the various institutes and in all maternal age and birthweight groups. Conversely, the incidence of associated congenital malformations was higher in the Negro (42.1 percent) than in the white (23.0 percent).

Associated congenital malformations were classified into three types: A (lethal or major), B (non-lethal), and C (borderline) malformations. The overall incidence of associated malformations (Types A, B, plus C) was 28.6 percent. When the Type C malformations were excluded, the incidence was 16.7 percent. Type A malformations were found in 9 of the 20 stillbirths and in 4 of the 6 neonatal deaths, but only in 7 (4.0 percent) of the 174 survivors. Skeletal system anomalies showed the highest incidence in the associated malformations. The incidence of SUA was 1.98 percent in infants of low birthweight (2,500 grams and under). In infants weighing over 2,500 grams the incidence was .63 percent.

Maternal diabetes was 6.4 percent, an incidence five times higher than in the control group.

The results of this study have been accepted for publication.

Part B included Yes

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Froehlich, Luz A. and Fujikura, Toshio: The Significance of a Single Umbilical Artery. American Journal of Obstetrics and Gynecology, In Press.